2. Aissa, J. et al: Molecular signaling at high dilution or by means of electronic circuitry. Journal of Immunology 150: A146, 1993

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ABSTRACTS
PART II

825

LONG-TERM CULTURE OF IL-3-DEPENDENT CELLS EXPRESSING *c-kit*, FcγRII/III AND p161 BUT LACKING FcεR. An FcεRI^{neg} MAST CELL? Carol Kinzer, Achsah Keegan, Marshall Plaut and William E. Paul. LI and Asthma and Allergy Branch, NIAID, Bethesda, MD 20892.

We have recently obtained a monoclonal antibody, K-1, that recognizes a 161 kDa glycoprotein expressed on the surface of murine mast cells but not on other mature hematopoietic cells. This antibody allows the sub-division of cell populations growing in cultures of bone marrow in response to IL-3. We observe that 7 to 10 days after initiation of such cultures, the predominant cell populations observed consist of 1) K-1^{pos}, c-kit^{pos}, FcεR^{pos} cells that are Alcian blue^{pos} and resemble mast cells; 2) K-1^{neg}, c-kit^{neg}, FcεR^{pos} cells that are enriched in cells with the morphology of murine basophils; and 3) K-1^{neg}, c-kit^{neg}, FcεR^{neg} cells. A small population of cells, 4), was also observed that were K-1^{pos}, FcεR^{neg}. All four cell populations were purified by electronic cell sorting and placed in culture with IL-3 for 3-6 weeks. At that time, populations 1 and 2 had given rise to K-1^{pos}, c-kit^{pos}, FcεR^{pos} that morphologically resembled mast cells. Interestingly, population 4 consisted largely of K-1^{pos}, FcεR^{neg} cells and population 3 had given rise to an approximately equal mixture of K-1^{pos}, FcεR^{pos} and K-1^{pos}, FcεR^{neg} cells. K-1^{pos} FcεR^{pos} and K-1^{pos}, FcεR^{neg} cells were re-purified by cell sorting and have been maintained in culture in IL-3 for greater than 9 months. These cells have retained their original character. For more detailed study, the cells have been expanded in IL-3 plus stem cell factor. The K-1^{pos}, FcεR^{neg} cells express *c-kit* and stain positively with 2.4G2, indicating that they express FcγRII or RIII. These FcεR^{neg} cell have the morphology of mast cells, contain Alcian blue positive granules and have a histamine content equivalent to that of the K-1^{pos} FcεR^{pos} cells (i.e. ~0.02 pg/cell), suggesting they represent FcεR^{neg} mast cells.

826

MAST CELLS EXPRESS AN ALTERNATIVELY SPLICED FORM OF PACAP, A NEUROPEPTIDE. E.V. Marietta and J.H.Weis. Division of Cell Biology and Immunology, Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT 84132

A sequence specific for the 5' untranslated region of the gene encoding Pituitary Adenylate Cyclase Activating Polypeptide (PACAP), a neuropeptide originally found in the hypothalamus, was identified in a screen of a rat mast cell cDNA library using a subtracted, mast cell enriched cDNA probe. Analysis of the PACAP transcripts produced in both rat and mouse brain, and from rat and mouse mast cells (RBL cell and IL-3 derived BMMC, respectively) indicated that mast cells express this gene. In addition, use of PCR primers flanking the coding sequences for the PACAP and the PRP related peptide indicated that the mast cells described above express an alternatively spliced form of the transcript. This site of alternative splicing is within the region encoding the mature PACAP protein. These data demonstrate that mast cells express the PACAP gene and that the resulting neuropeptide may be distinct from the previously described hypothalamus derived PACAP neuropeptide.

827

ANTIGEN PRESENTATION BY HIGHLY ENRICHED MAST CELL POPULATIONS. C. C. Fox and S. D. Jewell. The Ohio State University College of Medicine, Columbus, OH 43210.

Mast cells have been reported to express MHC class II molecules, but the significance of this has not been explored. In the present study, we examined whether purified Lewis rat peritoneal mast cells (MC) express MHC class II and accessory molecules and are capable of presenting antigen to antigen-specific T cell lines. MC were purified from peritoneal lavage to 98±0.1% purity using discontinuous Percoll density gradients. Contaminating cells (2%) consisted of lymphocytes, monocytes, eosinophils and neutrophils. By flow cytometric analysis, 6-57% (mean 24.1±18%, n=8) of MC expressed MHC class II antigen recognized by OX6 antibody. Virtually all MC expressed intracellular adhesion molecule-1 (ICAM-1) (97.8±2.8%) and LFA-1β (95.7±3.4%). Having determined that these MC express appropriate molecules for antigen presentation, they were then tested for their ability to present antigen to an autologous PPD antigen-specific T cell line (T cells), and compared with antigen presenting cells (APC) consisting of dispersed rat thymus cells. T cells were cultured with irradiated (3300r) APC or MC in the presence or absence of PPD for 72 hours, the last 18 hours of which included a pulse with [^{3}H]thymidine. MC antigen presentation resulted in 27-fold stimulation of T cell [^{3}H]thymidine incorporation, which was 40% of that induced by APC positive controls. This T cell proliferation was not the result of MC degranulation or cytokine release alone, since controls with MC and T cells without antigen showed negligible incorporation. While antigen presentation by the contaminating cells cannot be ruled out, these data suggest that mast cells express the appropriate molecules for and are capable of antigen presentation.

828

MURINE BONE MARROW DERIVED MAST CELLS ADHERE TO FIBRONECTIN FOLLOWING STIMULATION BY STEM CELL FACTOR. J. Dastych, P.J. Bianchine and D.D. Metcalfe. National Institutes of Health, Bethesda, MD 20892.

Bone marrow derived mast cells (MC) are known to adhere to fibronectin (FN) in detectable numbers after stimulation with phorbol ester (PE) or through $Fc_\epsilon RI$. Stem cell factor (SCF) (c-kit ligand) produced by stromal cells is known to promote MC proliferation and differentiation. We thus hypothesized that this factor could also be the activating signal for physiologic MC adhesion in tissues. To explore this question, we employed primary mouse bone marrow MC cultures in a series of adhesion experiments. Radiolabeled MC were seeded in plastic wells coated with FN in the presence and absence of SCF, and the percentage of cells remaining attached after rinsing with media determined. The addition of SCF promoted MC adhesion in a dose-response fashion. As little as 10 ng/ml increased adhesion from 4% to 52%. No adhesion was observed in plastic plates coated with bovine serum albumin only. Adhesion after SCF activation was completely abolished by 1mM EGTA. The FN peptide GRGDSP was able to inhibit 29 to 70% of cellular adhesion at concentrations between 500 to 1500 μg/ml. The control peptide GRGESP did not affect adhesion, nor did the peptide EILDVPST matching the alternative cell adhesion site on the FN molecule. Preincubation of cells with the tyrosine kinase inhibitor genistein resulted in partial inhibition of SCF-induced adhesion. We conclude that SCF prepares MC to adhere to FN in a process that is Ca dependent, occurs through the RGD binding site, and may involve a protein kinase. Because SCF is produced by stromal cells in tissues, SCF provides a physiological stimulus to MC to promote the engagement of MC to extracellular matrix.

829

A CLOSELY LINKED COMPLEX OF MOUSE MAST CELL-SPECIFIC CHYMASE GENES ON CHROMOSOME 14. M.F. Gurish, J.H. Nadeau, K.R. Johnson, H.P. McNeil, K.M. Grattan, K.F. Austen, and R.L. Stevens. Harvard Med. Sch., Boston, MA 02115, and the Jackson Lab., Bar Harbor, ME 04609.

Mouse mast cells differentially express four proteases [mouse mast cell protease (mMCP) 1, mMCP-2, mMCP-4, and mMCP-5] whose primary amino acid sequences are homologous to chymotrypsin, a protease (mMCP-6) homologous to trypsin, and an exopeptidase [mast cell carboxypeptidase A (MC-CPA)]. The previously uncharacterized 2.5-kb mMCP-2 gene was isolated and found to consist of 5 exons; its 5′ flanking region was 89%, 93%, and 42% homologous to that of the mMCP-1, mMCP-4, and mMCP-5 genes, respectively. Whereas the mMCP-6 and MC-CPA genes were located on chromosomes 17 and 3, respectively, the four mast cell protease genes all resided on chromosome 14 linked to a gene complex that encodes four cytotoxic T lymphocyte granzymes. Pulsed field gel electrophoresis demonstrated that the mMCP-1, mMCP-2, and mMCP-5 genes are within 850 kb of each another. We propose that a primordial gene that encoded a serine protease with restricted substrate specificity underwent extensive duplication and divergence to form a family of cytokine-regulated genes on chromosome 14. (Supported by NIH grants AI-22531, AI-23483, AI-31599, GM-46697, HG-00189, and HL-36110).

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MOLECULAR SIGNALING AT HIGH DILUTION OR BY MEANS OF ELECTRONIC CIRCUITRY. J. Aïssa, M.H. Litime, E. Attias and J. Benveniste. INSERM U 200, 32 rue des Carnets, 92140 Clamart, France.

We and others have previously reported biological effects of substances so highly dilute (HD) that no original molecule could be present. We have now improved the reliability of the response of isolated guinea-pig (GP) hearts (FASEB J. 1992, 6: 1610). Coronary flow variations (CFV) upon infusion of log 31-41"M" dilutions were greatest 1-3 wk after IP injection of ovalbumin (O) 1 ug in 0.1 ml alum (n = 30-77): buffer, 4.1 ± 0.5 (% mean ± SEM); histamine(H), 23.9 ± 2.0; O, 30.9 ± 4.0; (H, O: $p < 0.001$ from buffer). H, O (0.1 uM): 27.8 ± 4.2, 50.0 ± 3.5 respectively. HD O applied to hearts from non-immunized GP or submitted to a 50 Hz, 125 Oersted magnetic field (FASEB J. 1991, 5:1583) showed no activity. These data imply that molecules communicate via electromagnetic (EM) signals, thus transferrable by EM means. H, O, LPS (L), and water (W) in sealed vials were "transferred" to other W vials using an appropriately designed amplifier with input and output coils. Transferred W_W, W_H, W_O, W_L (n = 20-36, including two blind experiments supervised by external scientists) induced CFV of 8.8 ± 1.3, 33.6 ± 3.9, 34.4 ± 4.2, 39.9 ± 5.9 respectively (W_W vs others $p < 0.001$). These results confirm the EM nature of the molecular signal, presumably conveyed by polarized water dipoles associated with charged molecules (Phys. Rev. Lett. 1988, 61: 1085). They could radically change concepts of molecular signaling and provide new tools of intervention. Support: Dolisos Lab., Homint Lab., Bouygues and Science Innovante.

4. Benveniste, J. et al.: Transfer of molecular signal by electronic amplification. FASEB J. 8: A398, 1994

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ABSTRACTS

PART I

ABSTRACTS 1-3391

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2301

CONTINUOUS EXPOSURE OF RAT EMBRYOS TO A 1.5 G ELECTROMAGNETIC FIELD (EMF) DOES NOT AFFECT IN VITRO DEVELOPMENT AND VIABILITY. M. P. Dooley, M. H. Pineda, J. W. Lamont, R. J. Weber and D. J. Move (SPON. R. L. Engen). Depts. of Veterinary Physiology and Pharmacology and Electrical Engineering and Computer Engineering, Iowa State University, Ames, IA 50011

Some studies have associated residential and occupational exposures of humans to EMFs with increased incidence of cancer, impaired reproductive performance, and other forms of illness. Using a 60 Hz EMF generation system and shielded culture chamber developed in our laboratories, we determined the effects on in vitro development and viability of continuous exposure of rat embryos to a 1.5 G magnetic field for 72 h. The cleavage to blastocyst stage of 8-cell embryos exposed to an EMF of 1.5 G during culture, was not different ($P > 0.05$) from control embryos maintained at ≤ 0.005 G. The proportions of control (72/72; 100%) and treated (70/73; 96%) embryos that were viable at the end of the 72 h period of culture were not different ($P > 0.05$). Furthermore, the incidence of death of blastomeres was not different ($P > 0.1$) between embryos from control and treated groups. We conclude that acute, continuous exposure of rat embryos to an EMF of 1.5 G during in vitro culture does not affect the viability and developmental capacity of blastomeres. We propose that preimplantation embryos offer a mammalian model to study acute and delayed effects of exposure of cells to 60 Hz EMFs of defined intensity.

Supported by a grant from the Iowa Test and Evaluation Facility

2302

TRANSIENT ELECTROMAGNETIC FIELDS ALTER GROWTH RATE OF RABBIT SYNOVIOCYTES (HIG-82) *IN VITRO*. A.A. Marino, I.Iliev, J.P. Mains, F.X. Hart and R. Wolf. Dept. of Orthopaedic Surgery, LSU Medical Center, Shreveport, LA 71130

If HIG-82 cells in plastic flasks containing Dulbecco's modified Eagle media (DMEM) are exposed to an air electric field of 100 V/m having an instantaneous rise-time, the cells experience a media electric field of about 1 V/m for several nsec; however, if the air-field rise-time is 0.1 msec, the DMEM electric field does not depart from its equilibrium value of about 10^{-11} V/m. We applied pulsed electric fields having rise-times of either 1 nsec (E-fast) or 0.1 msec (E-slow) to HIG-82 cells, and measured the effect on cell proliferation. E-fast approximately doubled the growth rate after 1 and 2 days' exposure, but E-slow had no effect (N=12 in each group; Mean (10^6 cells/ml) ± SD).

	E-fast	Control	E-slow	Control	
1 Day	*7.4±0.8	4.1±1.0	3.6±.03	3.5±0.7	*P < 0.05,
2 Days	*18.7±0.6	9.3±0.9	11.6±0.6	11.5±0.4	t test

Cells exposed to E-fast for 1 day exhibited altered membrane-potential sensitivity to rhIL-1ß (20 ng/ml), suggesting a field-induced effect on responsiveness to growth factors. The results may explain some bioeffects of pulsed as compared with continuous fields.

Supported by Center of Excellence for Arthritis and Rheumatology

2303

FURTHER STUDIES OF ABNORMAL DEVELOPMENT OF JAPANESE QUAIL EMBRYOS EXPOSED TO HIGH LEVEL PULSED MAGNETIC FIELDS (PMF). R.O. Kelley and P.G. McGuire, University of New Mexico, Albuquerque, NM 87131 and C.A. Frost, Sandia National Laboratory, Albuquerque, NM 87185.

Introduction: We hypothesize that inductively coupled currents are established in cells of developing tissues and that these currents are the cause of the observed perturbations in embryonic development. To test this, we exposed embryonic cells in culture to PMF in each of two orientations. One orientation exposed cells to free flow of inductively coupled currents whereas the other blocked the flow.

Methods: High level PMF was produced using pulsed-power techniques to drive an air core solenoid coil with a thyristor switched capacitor discharge. The circuit was carefully designed to produce a smooth magnetic field waveform which was characterized with a B-dot loop sensor and displayed on a 100 MHz oscilloscope. The resulting magnetic field was a single cycle of 3 kHz sine wave with a peak value of 400 gauss.

Results: After three days of development, electron and light microscopy revealed structural perturbations in cells composing the basic embryonic tissues in embryos grown in high level PMF. After five days, experimental embryos showed a variety of abnormalities ranging from caudal regression to coloboma (failure of the intraocular fissure to close). Controls were normal. In addition, RNA fingerprinting suggests mRNA transcripts are differentially sensitive to the effect of the high level pulsed field.

Discussion: Observations of bioeffects in embryos grown in the high level PMF as early as the third day of development (during the initiation of major organ system development) suggests an effect at the cellular, and possibly the genetic, level. *In vitro* studies test the hypothesis that induced current coupling is the mechanism that perturbs normal embryonic development.

2304

TRANSFER OF THE MOLECULAR SIGNAL BY ELECTRONIC AMPLIFICATION. J. Benveniste, J. Aïssa, M.H. Litime, G.Th. Tsangaris, Y. Thomas. INSERM U 200, 32 rue des Carnets, 92140 Clamart, France.

An electromagnetic (EM) field abolished the activity of highly dilute ligands (FASEB J. 1991, 5:A1583), suggesting that they depend on EM fields. EM fields being in principle electronically transferable, we built an amplifier (gain: 100 µV/6 V and 100 nA/150 mA) fitted with one input and one output EM coil. In blind and open experiments, vials of ovalbumin (Ova, 10 nM), LPS (1 µg) or water (W) were placed on the input coil. Water vials (*Ova, LPS, W* respectively) were placed (15 min) on the output coil and then tested on isolated hearts from Ova-immunized guinea-pigs. Coronary flow variations (CFV) were (%, mean ± SEM, n=20-36): *Ova*, 26.6 ± 2.7; *LPS*, 26.1 ± 2.8; *W*, 6.2 ± 0.7 (*W vs Ova, LPS*: p≤e-8). In hearts from Ova-immunized rats, *Ova* induced (63 open exp.) 99.4 ± 11.7 % of the CFV induced by 0.1 µM Ova and 88.9 ± 9.4 in 24 blind exp. (*W* vs *Ova*, p=e-11). In addition, adrenaline activity was directly transferred, without *W* as intermediate, to human T-cells (CEM-C12 line) and cadmium was then added for 18 h. Cell survival (MTT test) was (%) 36.8 ± 5.0 *vs* 52.0 ± 7.4 (8 µM Cd^{2+}) and 21.6 ± 3.6 *vs* 34.0 ± 6.4 (10 µM Cd^{2+}) for transferred vehicle and adrenaline respectively (n=7, p<0.05). Thus the physical carrier of the molecular signal could be specific EM fields, possibly supported by polarized water dipoles in a state of "attractive interaction" (Phys. Rev. Lett. 1988, 61:1085). EM fields may be digitally processed, furnishing new tools for molecular medicine. (Supported by Dolisos Lab., Homint Lab., Bouygues SA and Assoc. Science Innovante).

2305

ACUTE 60-Hz MAGNETIC FIELD EFFECTS ON Ca^{2+} (Mn^{2+}) INFLUX IN JURKAT T-CELLS: STRICT DEPENDENCE ON CELL STATE. J. Walleczek, P.L. Killoran and W.R. Adey. Jerry L. Pettis Memorial Veterans Medical Center, Loma Linda, CA. 92357.

Electromagnetic field (EMF)-modulation of cellular signal transduction events, including Ca^{2+} signaling, is a reasonable candidate for mediating EMF effects such as on lymphocyte gene expression or proliferation [Walleczek (1992) FASEB J. 6: 3177-3185]. Thus, we studied acute 60-Hz EMF effects on Ca^{2+} (Mn^{2+})-transmembrane influx in FURA-2-loaded human Jurkat T-cells. The rate of Mn^{2+}-induced FURA-2 fluorescence quenching was used as a measure of Ca^{2+} influx rates, which were monitored by differential real-time fluorescence spectroscopy in the EMF-exposed and the control samples, *simultaneously*. We found that the biological state of the cell, as characterized by the pre-exposure Ca^{2+} influx rate, is a *predictor* of the magnitude of the 60-Hz MF effect (B_{rms} = 2 mT, f = 60 Hz sine, E_{max} = 18 µV/cm, t = 120 sec): No field effects (n = 65) were observed for cell preparations with initially high influx rates (> 40,000 pcs/min). In contrast, Ca^{2+} influx for T-cells with low pre-exposure flux rates (< 40,000 pcs/min) was significantly increased by 3.52% (n = 88; p = 0.0001) after a single 2-min exposure. Finally, the Ca^{2+} influx rate for T-cells with even lower pre-exposure flux rates (< 30,000 pcs/min) was enhanced even more by the 60-Hz EMF, namely by 5.0% (n = 37; p = 0.004). Supported by the Fetzer Institute and US-Department of Energy.

2306

SHIELDED CULTURE CHAMBER AND CONTROLLED UNIAXIAL MAGNETIC FIELD GENERATOR FOR VERY LOW FREQUENCY (VLF) MAGNETIC FIELD EXPOSURE OF CELLS DURING IN VITRO CULTURE. J. W. Lamont, R. J. Weber, M. P. Dooley, M. H. Pineda and D. J. Move (SPON. R. L. Engen). Depts. of Electrical Engineering and Computer Engineering and Veterinary Physiology and Pharmacology, Iowa State University, Ames, IA 50011

We have determined that incubators used for mammalian cell culture and in vitro fertilization in humans can generate VLF electromagnetic fields that vary in intensity and may exceed 800 milligauss. We describe a solenoidal magnetic field exposure system and in vitro incubation chamber which can expose cultured cells to uniaxial AC fields with control of the intensity, harmonics, and fundamental frequency of the magnetic field generated. To shield control cells or embryos during in vitro culture, a chamber of comparable dimensions was constructed using metals with high magnetic field permeability and attenuation features. The design and control features of the magnetic field generation system allow for the controlled exposure of embryos to time-varying, VLF magnetic fields ranging from background (≤ 5 milligauss) to 1500 milligauss. This system is currently being used to determine the effects of VLF (60 Hz) fields on the viability and developmental capacity of mammalian embryos.

Supported by a grant from the Iowa Test and Evaluation Facility

10. Citro, M. et al.: Hormon effects by electronic transmission. FASEB Journal 9: A392, 1995

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ABSTRACTS

PART I

ABSTRACTS 1-3621

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2268

DESENSITIZATION OF FUNCTIONAL G PROTEIN-COUPLED RECEPTORS MEASURED WITH A CYTOSENSOR MICROPHYSIOMETER. K. E. J. Dickinson, C. C. Bryson and S. Skwish (SPON: J. R. Powell). Dept. Cardiovascular Biochemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ, 08543.

The Cytosensor microphysiometer (Molecular Devices, Sunnyvale, CA) measures cellular acidification rates (CAR) using light addressable potentiometric sensor technology. Stimulation of LRM55b cells with catecholamines increased CARs via β_2-ARs. Iso-stimulated increase in CARs was inhibited by 50 μM (but not 10 μM) of EIPA (5-(N-ethyl-N-isopropyl)-amiloride) which suggests CARs were coupled to a Na/H exchanger. Desensitization of ß-AR mediated increases in CARs was shown by: sequentially decreased responses to repetitive applications of Iso and decreased responses to high concentrations of agonists. Rat aortic smooth muscle (RASM) cells contain endogenous receptors coupled to CARs. Time-dependent desensitization of endothelin (ET), angiotensin (AII), thrombin (THR) and bradykinin (BK) receptors was observed. Long agonist exposures (8-12 minutes) desensitized all receptors while shorter applications of AII and ET-1 (10 sec and 1 min respectively) allowed concentration-response curves to be obtained. Receptor desensitization was homologous. The specific ET antagonists SB-209670 and BMS-182874 dose-dependently inhibited the response to 1 nM ET-1 and protected the ET receptor from desensitization. The use of short application times allowed EC_{50} values for the ET and AII receptors to be determined (0.3±0.1 and 18±13 nM, respectively). K_B values for the ET receptor antagonists SB-209670 and BMS-182874 and the AII antagonist losartan were 1.2±0.3, 26±1.4 and 2.5±1.0 nM, respectively. EIPA (10 μM) inhibited ET, AII, BK, and THR mediated increases in CARs (82, 42, 70, and 50% respectively) which suggests that agonist-stimulated CARs involved Na/H exchanger. The Cytosensor Microphysiometer can be used to investigate receptor desensitization of G protein-coupled receptors and despite receptor desensitization, experimental protocols can be designed to obtain estimates of drug potency at physiologically relevant receptors.

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"CAGED" CARBON MONOXIDE: PHOTOLABILE MOLECULES THAT RELEASE FREE CARBON MONOXIDE UPON ILLUMINATION. Joseph P. Y. Kao, and Paul F. Keitz. Medical Biotechnology Center, and Dept. of Physiology, School of Medicine, Univ. of Maryland, Baltimore, MD 21201.

We report the design and chemical synthesis of a family of three "caged CO" compounds—photosensitive reagents that release the messenger molecule carbon monoxide when illuminated with long-wavelength ultraviolet light. The caged CO's are useful as Na^+ or K^+ salts, which can be bath-applied or introduced into cells via patch pipets, respectively. Alternatively, the caged CO's can be readily loaded into living cells by incubation with the acetoxymethyl (AM) ester forms of the reagents. Because the photosensitive "cages" in these compounds are based on o-nitrobenzyl chemistry—the most widely-used caging chemistry in biology—common mercury or xenon light sources are sufficient to activate the photorelease of carbon monoxide. Because photorelease offers the dual advantages of spatial and temporal control of messenger molecule release, these caged CO's are ideal for applications in signalling studies.

(Supported by NIH grant GM46956 and an SRIS Grant from the Univ. of MD School of Medicine)

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HORMONE EFFECTS BY CD RECORD /REPLAY.
F.Senekowitsch*, P.C.Endler**, W.Pongratz', C.W.Smith" (Spon.: Th.Kenner). *Inst.Bioinformatik, Graz, A, **Zoolog.U.Inst., Universitätsplatz 2, A-8010 Graz, Austria, 'LBI HOM, Graz, "U.Salford,UK.

Molecular bio-information may be transduced via water (1-4) or by means of an electronic device (5,6) and hence inhibit amphibian metamorphosis (6). Vials of thyroxine (T), E-30 M, (2) or water (W) were placed on an input coil linked to a filter and to an amplifier with a gain of E6. Frequencies below 80 kHz were digitized at the Nyquist frequency, buffered in a RAM and multiplexed on to a CD. After noise reduction and filtering the signal was attenuated by E6 to restore the original analog level. Water vials (WT, WW) were placed for 4 min on an output coil. WT or WW was added to the basin water of Amphibian larvae *R. temp.* at a 2-legged stage (2). Cumulative statistical frequencies of 4-legged stage F_a and of reduced tail F_b were evaluated. (WT: $N_{animals} = 234$; WW: $N_a = 234$). $F_{a,b}$ were (%, mean ± 1SD):

	WT1	WW1	WT2	WW2	WT3	WW3	WT4	WW4
F_a:	40±08	54±07	53±09	65±08	64±08	73±08	74±08	86±09
F_b:	24±10	30±08	28±13	39±07	46±08	56±06	58±12	69±06

1-4, depending on the experiment: intervals of 24-48 h. Comparison WT *vs* WW, $p < 0.001$ in chi-square test and t-test. Data also significant in "survival analysis".

Water dipoles may develop phase coherent oscillations through radiation coupling (7), it is proposed that these could be modulated as a time-ordered pattern of signals (2) and induce electron propagation (2).

(1) Benveniste et al. FASEB J. 1991,5:A1008;A1538. 1992,6:A1610; Youbicier-Simo et al. Int.J.Imun. 1993,IX:169; Endler et al. J.Vet.Hum.Tox. 1994,36:56. (2) Endler /Schulte, Dordrecht: Kluwer 1994. (3) Smith. Neural Network 1994,3:379. (4) Van Wijk, Smith in Ref. 2; Endler et al. FASEB J. 1994,8:A400. (5) Aissa et al. J. Immunol. 1993,150:A146; Benveniste et al. FASEB J. 1994,8:A398. (6) Citro et al. in Ref. 2. (7) Del Giudice / Preparata Phys.Rev.Lett. 1988,61:1085; Aissa et al. FASEB J. 1993,7:A602.

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HORMONE EFFECTS BY ELECTRONIC TRANSMISSION. M.Citro*, P.C.Endler**, W.Pongratz***, C.Vinattieri', C.W.Smith", J.Schulte'" (Spon.:Th.Kenner). *IDRAS,I,**Zoolog.U.Inst.,Universitätsplatz 2, A-8010 Graz,Austria,***LBI HOM,A,'U.Urbino,I,"U.Salford,UK,"MSU, USA.

Molecular bio-information may be transduced via water (1-3) and exert bio-activity even when the water is within hard-glass vials (4). This may be transferred by means of an electronic device (5) and hence inhibit amphibian metamorphosis (6). Vials of thyroxine (T) (1 mM) or water (W) were placed on the input coil of a special amplifier (linear from DC to HF). Water vials (WT, WW) were placed for 4 min on the output coil. WT or WW was added to the basin water of Amphibian larvae *R. temp.* at a 2-legged stage (2). Cumulative statistical frequencies of 4-legged stage F_a and of reduced tail F_b were evaluated at intervals of 8 h (6). The experiment was now repeated in two laboratories (A, I). WT: $N_{animals} = 468$; WW: $N_a = 468$. $F_{a,b}$ were (%, mean ± 1 SD):

	WT1	WW1	WT2	WW2	WT3	WW3	WT4	WW4
F_a:	31±12	44±11	53±12	66±13	65±12	80±15	77±13	89±11
F_b:	27±11	43±13	37±12	52±12	49±14	63±09	62±16	73±09

1-4, depending on the experiment: intervals of 8-80 h. Comparison WT *vs* WW, $p < 0.001$ in chi-square test and t-test. Data also significant in "survival analysis".

Diluent water (and other polar) molecules may undergo phase coherent oscillations through radiation coupling (7), that are speculated to induce electron propagation (2).

(1) Benveniste et al. FASEB J. 1991,5:A1008; A1538. 1992,6:A1610; Youbicier-Simo et al. Int.J.Immunotherapie 1993,IX:169; Endler et al. J.Vet.Human Tox. 1994,36:56. (2) Endler and Schulte, Dordrecht: Kluwer 1994. (3) Smith. Neural Network 1994,3:379. (4) Van Wijk, Smith in Ref. 2; Endler et al. FASEB J. 1994,8:A400. (5) Aissa et al. J. Immunol. 1993,150:A146; Benveniste et al. FASEB J. 1994,8:A398. (6) Citro et al. in Ref. 2. (7) Del Giudice and Preparata Phys.Rev.Lett. 1988,61:1085; Aissa et al. FASEB J. 1993,7:A602; Del Giudice in Ref 2. (Supported by Brügemann Institute, FRG.)

2272

Detection of Femtomolar Levels of Diacylglycerol Molecular Species via Gas Chromatograpy-Mass Spectroscopy W.C. Hubbard, T.R. Hundley and D.W. MacGlashan, Jr. Johns Hopkins Asthma & Allergy Center, Baltimore, MD 21224.

Diacylglycerols (DAG) play a role in early receptor-mediated signal transduction events in many cells. While the identity and rate of formation of molecular species of DAG in cells are critical in determination of the role of these lipids in signal transduction, conventional assays lack adequate sensitivity and selectivity for measurement of femtomolar levels of released DAG molecular species in cells particularly when the availability of cells is limited. We have developed a highly selective technique which is 100 fold more sensitive than conventional techniques. The method employs combined gas chromatography-negative ion chemical ionization mass spectrometry (GC-MS) for detection of DAG molecular species by monitoring molecular anions. Extracted DAG are converted to a pentafluorobenzoyl ester derivatives prior to GC-MS analysis. In pilot studies with 1-stearoyl-2-arachidonoyl-3-glycerol we have shown that the assay met-hod displays a broad linear range with a sensitivity of <15 femtomoles. The assay method is highly reproducible with assay inter- and intra-variations of <15%. Presently, this protocol is being used for the profiling of DAG molecular species released by basophils and mast cells during receptor-mediated activation. For example, preliminary studies examining only the generation of 1-stearoyl-2-arachidonoyl-3-glycerol indicate that there are resting levels of 2-5 pmoles/10^6 of this species (compared to 15-20 pmoles/10^6 total DAG as measured by a DAG kinase assay) in human basophils which, in the first 30 seconds, increased only 50-100% following stimulation with formyl-met-leu-phe.

11. Senekowitsch, F. et al.: Hormone effects by CD record/replay. FASEB Journal 9: A392, 1995

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ABSTRACTS

PART I

ABSTRACTS 1-3621

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2268

DESENSITIZATION OF FUNCTIONAL G PROTEIN-COUPLED RECEPTORS MEASURED WITH A CYTOSENSOR MICROPHYSIOMETER. K. E. J. Dickinson, C. C. Bryson and S. Skwish (SPON: J. R. Powell). Dept. Cardiovascular Biochemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ, 08543.

The Cytosensor microphysiometer (Molecular Devices, Sunnyvale, CA) measures cellular acidification rates (CAR) using light addressable potentiometric sensor technology. Stimulation of LRM55b cells with catecholamines increased CARs via β_2-ARs. Iso-stimulated increase in CARs was inhibited by 50 μM (but not 10 μM) of EIPA (5-(N-ethyl-N-isopropyl)-amiloride) which suggests CARs were coupled to a Na/H exchanger. Desensitization of ß-AR mediated increases in CARs was shown by: sequentially decreased responses to repetitive applications of Iso and decreased responses to high concentrations of agonists. Rat aortic smooth muscle (RASM) cells contain endogenous receptors coupled to CARs. Time-dependent desensitization of endothelin (ET), angiotensin (AII), thrombin (THR) and bradykinin (BK) receptors was observed. Long agonist exposures (8-12 minutes) desensitized all receptors while shorter applications of AII and ET-1 (10 sec and 1 min respectively) allowed concentration-response curves to be obtained. Receptor desensitization was homologous. The specific ET antagonists SB-209670 and BMS-182874 dose-dependently inhibited the response to 1 nM ET-1 and protected the ET receptor from desensitization. The use of short application times allowed EC_{50} values for the ET and AII receptors to be determined (0.3±0.1 and 18±13 nM, respectively). K_B values for the ET receptor antagonists SB-209670 and BMS-182874 and the AII antagonist losartan were 1.2±0.3, 26±1.4 and 2.5±1.0 nM, respectively. EIPA (10 μM) inhibited ET, AII, BK, and THR mediated increases in CARs (82, 42, 70, and 50% respectively) which suggests that agonist-stimulated CARs involved Na/H exchanger. The Cytosensor Microphysiometer can be used to investigate receptor desensitization of G protein-coupled receptors and despite receptor desensitization, experimental protocols can be designed to obtain estimates of drug potency at physiologically relevant receptors.

2269

"CAGED" CARBON MONOXIDE: PHOTOLABILE MOLECULES THAT RELEASE FREE CARBON MONOXIDE UPON ILLUMINATION. Joseph P. Y. Kao, and Paul F. Keltz. Medical Biotechnology Center, and Dept. of Physiology, School of Medicine, Univ. of Maryland, Baltimore, MD 21201.

We report the design and chemical synthesis of a family of three "caged CO" compounds—photosensitive reagents that release the messenger molecule carbon monoxide when illuminated with long-wavelength ultraviolet light. The caged CO's are useful as Na^+ or K^+ salts, which can be bath-applied or introduced into cells via patch pipets, respectively. Alternatively, the caged CO's can be readily loaded into living cells by incubation with the acetoxymethyl (AM) ester forms of the reagents. Because the photosensitive "cages" in these compounds are based on *o*-nitrobenzyl chemistry—the most widely-used caging chemistry in biology—common mercury or xenon light sources are sufficient to activate the photorelease of carbon monoxide. Because photorelease offers the dual advantages of spatial and temporal control of messenger molecule release, these caged CO's are ideal for applications in signalling studies.

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2270

HORMONE EFFECTS BY CD RECORD /REPLAY. F.Senekowitsch*, P.C.Endler**, W.Pongratz', C.W.Smith" (Spon.: Th.Kenner). *Inst.Bioinformatik, Graz, A, **Zoolog.U.Inst., Universitätsplatz 2, A-8010 Graz, Austria, 'LBI HOM, Graz, "U.Salford,UK.

Molecular bio-information may be transduced via water (1-4) or by means of an electronic device (5,6) and hence inhibit amphibian metamorphosis (6). Vials of thyroxine (T), E-30 M, (2) or water (W) were placed on an input coil linked to a filter and to an amplifier with a gain of E6. Frequencies below 80 kHz were digitized at the Nyquist frequency, buffered in a RAM and multiplexed on to a CD. After noise reduction and filtering the signal was attenuated by E6 to restore the original analog level. Water vials (WT, WW) were placed for 4 min on an output coil. WT or WW was added to the basin water of Amphibian larvae *R. temp.* at a 2-legged stage (2). Cumulative statistical frequencies of 4-legged stage F_a and of reduced tail F_b were evaluated. (WT: $N_{animals} = 234$; WW: $N_a = 234$). $F_{a,b}$ were (%, mean ± 1SD):

	WT1	WW1	WT2	WW2	WT3	WW3	WT4	WW4
F_a:	40±08	54±07	53±09	65±08	64±08	73±08	74±08	86±09
F_b:	24±10	30±08	28±13	39±07	46±08	56±06	58±12	69±06

1-4, depending on the experiment: intervals of 24-48 h . Comparison WT *vs* WW, $p < 0.001$ in chi-square test and t-test. Data also significant in "survival analysis".

Water dipoles may develop phase coherent oscillations through radiation coupling (7), it is proposed that these could be modulated as a time-ordered pattern of signals (2) and induce electron propagation (2).

(1) Benveniste et al. FASEB J. 1991,5:A1008;A1538. 1992,6:A1610; Youbicier-Simo et al. Int.J.Imun. 1993,IX:169; Endler et al. J.Vet.Hum.Tox. 1994,36:56. (2) Endler /Schulte, Dordrecht: Kluwer 1994. (3) Smith. Neural Network 1994,3:379. (4) Van Wijk, Smith in Ref. 2; Endler et al. FASEB J. 1994,8:A400. (5) Aissa et al. J. Immunol. 1993,150:A146; Benveniste et al. FASEB J. 1994,8:A398. (6) Citro et al. in Ref. 2. (7) Del Giudice / Preparata Phys.Rev.Lett. 1988,61:1085; Aissa et al. FASEB J. 1993,7:A602.

2271

HORMONE EFFECTS BY ELECTRONIC TRANSMISSION. M.Citro*, P.C.Endler**, W.Pongratz***, C.Vinattieri', C.W.Smith", J.Schulte"' (Spon.:Th.Kenner).*IDRAS,I,**Zoolog.U.Inst.,Universitätsplatz 2, A-8010 Graz,Austria,***LBI HOM,A,'U.Urbino,I,"U.Salford,UK,"MSU, USA.

Molecular bio-information may be transduced via water (1-3) and exert bio-activity even when the water is within hard-glass vials (4). This may be transferred by means of an electronic device (5) and hence inhibit amphibian metamorphosis (6). Vials of thyroxine (T) (1 mM) or water (W) were placed on the input coil of a special amplifier (linear from DC to HF). Water vials (WT, WW) were placed for 4 min on the output coil. WT or WW was added to the basin water of Amphibian larvae *R. temp.* at a 2-legged stage (2). Cumulative statistical frequencies of 4-legged stage F_a and of reduced tail F_b were evaluated at intervals of 8 h (6). The experiment was now repeated in two laboratories (A, I). WT: $N_{animals} = 468$; WW: $N_a = 468$. $F_{a,b}$ were (%, mean ± 1 SD):

	WT1	WW1	WT2	WW2	WT3	WW3	WT4	WW4
F_a:	31±12	44±11	53±12	66±13	65±12	80±15	77±13	89±11
F_b:	27±11	43±13	37±12	52±12	49±14	63±09	62±16	73±09

1-4, depending on the experiment: intervals of 8-80 h. Comparison WT *vs* WW, $p<0.001$ in chi-square test and t-test. Data also significant in "survival analysis".

Diluent water (and other polar) molecules may undergo phase coherent oscillations through radiation coupling (7), that are speculated to induce electron propagation (2).

(1) Benveniste et al. FASEB J. 1991,5:A1008; A1538. 1992,6:A1610; Youbicier-Simo et al. Int.J.Immunotherapie 1993,IX:169; Endler et al. J.Vet.Human Tox. 1994,36:56. (2) Endler and Schulte, Dordrecht: Kluwer 1994. (3) Smith. Neural Network 1994,3:379. (4) Van Wijk, Smith in Ref. 2; Endler et al. FASEB J. 1994,8:A400. (5) Aissa et al. J. Immunol. 1993,150:A146; Benveniste et al. FASEB J. 1994,8:A398. (6) Citro et al. in Ref. 2. (7) Del Giudice and Preparata Phys.Rev.Lett. 1988,61:1085; Aissa et al. FASEB J. 1993,7:A602; Del Giudice in Ref 2. (Supported by Brügemann Institute, FRG.)

2272

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Diacylglycerols (DAG) play a role in early receptor-mediated signal transduction events in many cells. While the identity and rate of formation of molecular species of DAG in cells are critical in determination of the role of these lipids in signal transduction, conventional assays lack adequate sensitivity and selectivity for measurement of femtomolar levels of released DAG molecular species in cells particularly when the availability of cells is limited. We have developed a highly selective technique which is 100 fold more sensitive than conventional techniques. The method employs combined gas chromatography-negative ion chemical ionization mass spectrometry (GC-MS) for detection of DAG molecular species by monitoring molecular anions. Extracted DAG are converted to a pentafluorobenzoyl ester derivatives prior to GC-MS analysis. In pilot studies with 1-stearoyl-2-arachidonoyl-3-glycerol we have shown that the assay met-hod displays a broad linear range with a sensitivity of <15 femtomoles. The assay method is highly reproducible with assay inter- and intra-variations of <15%. Presently, this protocol is being used for the profiling of DAG molecular species released by basophils and mast cells during receptor-mediated activation. For example, preliminary studies examining only the generation of 1-stearoyl-2-arachidonoyl-3-glycerol indicate that there are resting levels of 2-5 pmoles/10^6 of this species (compared to 15-20 pmoles/10^6 total DAG as measured by a DAG kinase assay) in human basophils which, in the first 30 seconds, increased only 50-100% following stimulation with formyl-met-leu-phe.

3. Aissa, J. et al.: Electronic transmission of the cholinergic signal. FASEB
Journal (Journal of the Federation of American Societies for Experimental
Biology) 9: A683, 1995

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ABSTRACTS

PART II

ABSTRACTS 3622-6187
Tutorials T1-T30

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3960

INCREASED RENAL SYMPATHETIC NERVE ACTIVITY (RSNA) AND MEAN ARTERIAL PRESSURE (MAP) FOLLOWING SYSTEMIC MCN-A-343. J. R. Martin, Kirksville Col. Osteo. Med., Kirksville, MO 63501

Intravenous (iv) administration of the muscarinic M_1 receptor agonist McN-A-343 (McN) to anesthetized, pithed rats evokes an increase in MAP which can be blocked by α-adrenergic receptor antagonists. The present study was undertaken to determine if McN can increase: 1) the MAP of conscious rats, and 2) RSNA of anesthetized rats. Sprague-Dawley rats (280-320 g) were implanted with catheters in the femoral artery and vein for blood pressure monitoring and drug administration. All rats were pretreated with the ganglionic nicotinic receptor antagonist pentolinium (10 mg/kg iv) to prevent baroreflex-mediated bradycardia. In conscious rats, McN evoked a dose-dependent increase in MAP. Determination and correlation of pA_2 values to known K_D values for five muscarininc receptor antagonists showed that the McN-evoked pressor effect resulted from activation of M_1 receptors. Benextramine, yohimbine, and propranolol, or adrenal demedullation coupled with chronic guanethidine treatment inhibited the McN-evoked increase in MAP. In urethane-anesthetized rats McN evoked a dose-dependent increase in MAP and RSNA which were attenuated by telenzepine (68 μg/kg iv). Addition of prazosin (0.2 mg/kg iv), but not desipramine (0.3 mg/kg iv), to the telenzepine pretreatment completely inhibited the McN-evoked increase in MAP. These results show that McN-A-343 increases MAP of conscious rats by increasing postganglionic sympathetic nerve activity. (Supported by NIH HL44531).

3961

MODULATION OF CHOLINERGIC TRANSMISSION BY DOPAMINERGIC AGENTS IN THE ISOLATED FIELD-STIMULATED GUINEA PIG ILEUM. M.A. Elwan, A.M. Ghazal, M.F. Sharabi and Magdi R.I. Soliman. College of Pharmacy, Florida A&M University, Tallahassee, Fl 32307.

The present study was conducted to investigate the possible modulation of peripheral cholinergic transmission by dopaminergic drugs. Guinea pigs of either sex were sacrificed, the ileum was isolated, cut into segments 2.5-3 cm long, set in 20 ml capacity tissue bath in Krebs solution at 37°C, oxygenated with 95% O_2 and 5% CO_2. A force-displacement transducer connected to Grass-7PD polygraph was used to record muscle contractions. Electrical stimulation was achieved by setting the muscle between two platinum electrodes connected to Grass-S48 stimulator. After 1 hr equilibration period, the muscle was stimulated by pulses of 30 V, 1 ms duration, 0.1 Hz. The contractions produced were of cholinergic nature, since it was inhibited by atropine ($3x10^{-8}$M). Dopamine (DA, $1.32x10^{-4}$M-$8.4x10^{-3}$M), apomorphine (APO, $4.1x10^{-6}$M-$6.6x10^{-5}$M) and bromocriptine (BROM, $2.6x10^{-6}$M-$4.3X10^{-5}$M), dose-dependently abolished the electrically-induced muscle contractions, suggesting the involvement of dopaminergic receptors. However, DA, APO or BROM, in the same concentrations, failed to inhibit muscle contraction induced by addition of exogenous acetylcholine (Ach, $2.8x10^{-7}$M), thus, ruling out any competition with Ach for the muscarinic receptors. Dopamine, APO and BROM inhibited the muscle response to the ganglion stimulant nicotine (NIC, $1.1x10^{-6}$M) indicating the possibility of exerting their action on the parasympathetic postganglionic nerve. This was confirmed by the observation that, DA still produced its inhibitory effect even in the presence of the ganglion blocker hexamethonium ($1.83x10^{-5}$M) which almost completely abolished NIC-induced responses. Dopamine, APO or BROM inhibitory effect on electrically-induced contractions was attenuated in the presence of dopaminergic receptor antagonists metoclopramide ($5X10^{-6}$M) or (±)-sulpiride ($5x10^{-5}$M) providing further evidence for dopaminergic receptors involvement. The present findings provide proof for the presence of dopaminergic receptors on the postganglionic parasympathetic nerve. These receptors may modulate cholinergic activity in presynaptic nerve terminals.

3962

Effect of caffeine and choline administration on the release of acetylcholine in awake rats by microdialysis.

J.P. Myers and D. A. Johnson (SPON: T. Maher) Department of Pharmacology-Toxicology, Schoool of Pharmacy, Duquesne University, Pittsburgh, PA 15282

This study was designed to show the effect of peripheral administration of caffeine, choline, or a combination of caffeine with choline on release of acetylcholine (ACh) from the hippocampus of conscious rats. An *in vivo* microdialysis technique was used to determine ACh release in the CA1 region of the hippocampus (A:-3.8, L:1.6, V:-3.8 from Bregma). Microdialysis probes were constructed with 6000 MW cutoff dialysis membrane having an effective length of 3.8 mm. Implanted probes were perfused with an artificial cerebrospinal fluid (CSF) at 3.0 ul/min. After an 18-hour recovery period, fractions of the dialysate were collected every 20 minutes. For each animal, three baseline samples were collected followed by the IP administration of drug. Subsequently, four samples were collected. ACh concentrations were determined using a liquid chromatograph with an electro-chemical detector. The ACh concentrations of the post-treatment samples were compared as a percentage to the ACh concentrations of the baseline samples for each animal. It was found that the administration of a combination of caffeine (10 mg/kg) with choline (120 mg/kg) caused a significant increase of 242%. The drugs administered alone produced no significant effect on ACh release.

3963

RESPONSE TO SELECTION FOR DIFFERENTIAL NICOTINE-INDUCED LOCOMOTOR ACTIVITY IN MICE. Toni Ness Smolen, Andrew Smolen and Joseph E. Berta. Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309-0447.

Mice are being selectively bred for differential response to a 0.75 mg/kg dose of nicotine using locomotor activity in an automated Y-maze. Nicotine/saline regression residuals are used to control for saline baseline activity. Starting from a Heterogeneous Stock of mice, duplicate Nicotine Depressed (ND), Nicotine Activated (NA), and Control lines have been produced. Since a previous study using within-family selection produced limited response in the NA lines, the present study employed mass selection.

After four generations, the response to selection has been very good. The differential responses of the ND and NA lines to nicotine has increased each generation, while saline baseline scores are nearly identical. Heritabilities are approximately 0.12 for the NA lines and 0.21 for the ND lines across all four generations. Several correlated responses, including density, affinity and genotype of brain nicotinic receptors, nicotine-induced corticosterone responses and nicotine pharmacokinetics are being monitored during the course of selection. Although the lines do not differ for these parameters as yet, it is expected that as selection proceeds, one or more will be found to be correlated with the behavioral response to nicotine. Supported by DA06330.

3964

ELECTRONIC TRANSMISSION OF THE CHOLINERGIC SIGNAL. J. Aïssa, P. Jurgens, M.H. Litime, I. Béhar and J. Benveniste. INSERM U 200, 32 rue des Carnets, 92140 Clamart, France.

Since 1992 we have transferred to water (W) [1-5] or directly to cells [6] *circa* 30 molecular signals via electronic circuitry. Acetylcholine (ACh), nicotine (Nic) and W transferred to W (***ACh, Nic, W***) were infused to isolated guinea-pig (P) or rat (R) hearts receiving the ACh-esterase (AChE) inhibitor eserine (2 mg/l). Coronary flow variations (CFV) were, in %, mean ± SEM (*n*): ***ACh*** 17.1 ± 1.0 (***49***), ***Nic*** 22.6 ± 2.2 (***9***), ***W*** 5.2 ± 0.3 (***66***), ponderal ACh (0.1 μM) 21.0 ± 1.6 (***42***) and Nic (0.1 μM) 15.3 ± 2.2 (***4***).

ACh was infused, with and w/o eserine (e), in hearts from naive P (NP, NPe) or ovalbumin-immunized P [1, 2] (OP, OPe) or from naive R (NR, NRe):

	NP (*19*)	NPe (*48*)	OP (*28*)	OPe (*49*)	NR (*24*)	NRe (*15*)
% values < 100 %	42*	15	14	8	25	0
% values > 100 %	58 (0.32)†	85 (3e-7)	86 (9e-5)	92 (4e-10)	75 (0.04)	100 (3e-5)
Mean ± S.E.M.	136 ± 21	310 ± 38	296 ± 33	284 ± 20	260 ± 41	383 ± 41

* Ratio % CFV ***Ach*** / % CFV ***W*** x 100. † p values: Binomial probability.

The null hypothesis (equal nb of values > and < 100 %) can be rejected for all groups but NP. Being transferred electronically, ***ACh*** appears electromagnetic in nature. Its potentiation by eserine, known to potentiate ACh, suggests that ***ACh*** interacts with both ACh receptors and molecular AChE. As we predicted [1-3], molecular signals have recently been digitized and recorded [5].

[1] Aïssa et al., FASEB J. 1993, 7:A602. [2] Benveniste et al., FASEB J. 1994, 8:A398. [3] Aïssa et al., [4] Citro et al., [5] Senekowitsch et al., [6] Thomas et al., this meeting.

Supported in part by Bouygues SA, SAUR and Association Science Innovante.

12. Thomas, Y. et al.: Direct Transmission to cells of a molecular signal
(phorbol myristate acetate, PMA) via an electronic device.. FASEB Journal 9:
A227. 1995

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ABSTRACTS

PART I

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1317

ARF TRANSLOCATION CORRELATES WITH POTENTIATION OF GTPγS-SENSITIVE PHOSHOLIPASE D (PLD) ACTIVITY IN MEMBRANE FRACTIONS OF HL-60 CELLS. M.G. Houle, P.H. Naccache and S. Bourgoin. Centre de Recherche en Rhumatologie et Immunologie, Laval Univ., Québec, G1V 4G2, Canada.

PLD catalyses the hydrolysis of phoshatidylcholine into choline and phosphatidic acid. To date, little is known about the regulation of this enzymatic activity. Recently the small molecular weight GTP-binding protein ARF has been implicated in PLD regulation. Pretreatment of HL-60 cells with phorbol esters [phorbol 12-myristate 13-acetate (PMA) or phorbol 12-dibutyrate (PdBu)] or with the chemoattractant fMet-Leu-Phe (fMLP) increases the GTPγS-stimulated PLD activity of membrane fractions derived from these cells. To verify whether ARF was the component involved in the potentiation of the GTPγS-stimulated PLD activity, ARF1 and ARF4 N-terminal peptides were incubated with membrane fractions derived from HL-60 cells pretreated with PMA. We obtained an inhibition of GTPγS-stimulated PLD activity which exceeded 50% with either peptides. Peptides derived from other G proteins or from irrelevant proteins were without effect. By Western blotting, using an anti-ARF antibody, followed by densitometric analysis, we observed an increase in the membrane content of ARF in cells treated with either phorbol esters or with fMLP. The inactive analogue of PdBu (4αPdBu) had no effect on the membrane content of ARF or on the GTPγS-stimulated PLD activity. We conclude that ARF translocation correlates with PLD activation by phorbol esters or by fMLP in HL-60 cells.

Supported by the National Cancer Institute and the Medical Research Council of Canada.

1318

MEMBRANE-ASSOCIATED PHOSPHOLIPASE D (PLD) IS REGULATED BY TWO CYTOSOLIC COMPONENTS IN HL60 CELLS. S. Bourgoin, Y. Desmarais, D. Harbour and A.D. Beaulieu (SPON: P.H. Naccache). Centre de Recherche en Rhumatologie et Immunologie, Laval Univ., Québec, G1V 4G2, Canada.

In granulocytes the activation of PLD by guanine nucleotides requires protein cofactors in both the plasma membrane and the cytosol. The active cytosolic component(s) was recovered in a 35-75% ammonium sulfate precipitate. The reconstitutively active fraction was further resolved on a size-exclusion gel. Two eluted protein fractions were both found to reconstitute the GTPγS-stimulated PLD activity when combined with [^{3}H]labeled HL60 membranes. The major peak of PLD-reconstituting activity was localized in a protein fraction enriched in 50-kDa proteins. The stimulatory GTP/GDP exchange factor, *smg* GDS, and the inhibitory GTP/GTP exchange factor, *rho* GDI, which are active on the *rho* family of small GTPases could be partially separated from the 50-kDa PLD-inducing factor(s). A second minor peak of PLD reconstitution was observed in the 18-kDa region. The 18-kDa peak of reconstituting activity was enriched in ARF small GTP-binding proteins. Small amounts of membrane-associated ARF were also detected. Stimulation by the 50-kDa and the 18-kDa components was synergistic. Moreover, the synthetic peptide of the N-terminus of ARF1(2-17) inhibits GTPγS-stimulated PLD activity in HL60 postnuclear homogenates. It is proposed that the 50-kDa cytosolic factor facilitates the PLD-stimulating function of ARF small GTPases.

Supported by the National Cancer Institute of Canada.

1319

Induction of macrophage (MΦ) procoagulant activity by murine hepatitis virus strain 3:The role of tyrosine phosphorylation

A.P.B. Dackiw, K. Zackrzewski, G.A. Levy and O.D. Rotstein.
Departments of Surgery and Medicine, University of Toronto, Toronto, Canada.

Fulminant hepatitis induced by murine hepatitis virus is characterized by the presence of sinusoidal thrombosis. Several lines of evidence support the concept that viral induced MΦ mediated fibrin deposition (procoagulant activity; PCA) plays a role in this disease process via expression of the virus induced procoagulant mouse fibrinogen-like protein (musfiblp) gene. Recent studies have reported that induction of tyrosine phosphorylation in MΦs by a variety of stimuli is a central signaling event leading to the generation of MΦ products. Thus, the role of tyrosine phosphorylation leading to MHV-3 induced procoagulant expression was studied. Murine peritoneal MΦs were incubated in the presence or absence of MHV-3. In some experiments cells were pretreated with tyrosine kinase inhibitors genistein (10μg/ml), herbimycin A (1μg/ml) or tyrphostin (25μg/ml). Cells were freeze thawed for measurement of PCA. PCA was examined by the ability of MΦs to shorten the clotting time of recalcified plasma. Tyrosine phosphorylation was determined by immunoblotting with anti-phosphotyrosine antibodies. Induction of musfiblp mRNA was assessed by northern blot analysis using a cDNA probe for musfiblp. **Results:** MHV-3 induced a marked increase in MΦ PCA. Tyrosine kinase inhibition resulted in functional inhibition of MΦ PCA. MHV-3 induced a time dependent increase in phosphorylated tyrosine residues by western blot analysis. Both genistein and herbimycin significantly reduced the level of phosphotyrosine accumulation without affecting cell viability. Genistein completely abrogated the MHV-3 stimulated rise in the level of musfiblp mRNA transcripts. **Thus:** Tyrosine phosphorylation plays a role in the signaling pathway leading to the induction of MΦ PCA by MHV-3. Since the expression of MΦ PCA correlates with the lethality associated with MHV-3 induced viral hepatitis, a novel approach to the treatment of this disease is suggested.

1320

DIRECT TRANSMISSION TO CELLS OF A MOLECULAR SIGNAL (PHORBOL MYRISTATE ACETATE, PMA) VIA AN ELECTRONIC DEVICE. Yolène Thomas, Michel Schiff,* M. Hedi Litime, Laurent Belkadi and Jacques Benveniste. INSERM U 200, 32 rue des Carnets, 92140 Clamart, and *CRHST-CNRS, Paris, France.

Molecular signals can be transmitted to water via electronic devices [1-5]. We investigated the effect of PMA, transmitted directly to isolated human neutrophils (transmitted PMA, vehicle: ***PMA, vehicle***), on superoxide production measured by cytochrome c reduction. PMA (1 μM) or vehicle tubes were random-coded and placed on the input coil of a specially designed electronic device. Cells (10^6) were placed on (or, as another control, beside) the output coil for 15 min at 37°C, and were then incubated for an additional 45 min at 37°C. Results are calculated as nmol $O_2^-/10^6$ cells/1 hr and, given the variable cell response to PMA or ***PMA***, expressed as %: $\Delta O_2^- = 100 \times [(PMA - vehicle) / vehicle]$. In 20 consecutive blind experiments, ΔO_2^- was 37 ± 6 % (mean ± S.E.M., $p < 0.001$) and controls yielded similar results as ***vehicle***. The effect of PMA and ***PMA*** was inhibited by superoxide dismutase (n = 3). Cell-containing tubes, wrapped or not in metal foil designed for magnetic shielding, were placed on the output coil during PMA transmission (n = 9). ΔO_2^- was 41 to 100 % and -29 to 17 % for unshielded and shielded tubes respectively. These results suggest direct PMA-like cell activation most likely via an electromagnetic signal.

[1] Aïssa et al., FASEB J. 1993, 7:A602. [2] Aïssa et al., this meeting. [3] Benveniste et al., FASEB J. 1994, 8:A398. [4] Citro et al., [5] Senekowitsch et al., this meeting.

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Altered Granulocyte H_2O_2 Release Following Severe Thermal Injury.

A.C. Drost, R. Moore, A.D. Mason, Jr., B.A. Pruitt, Jr., and W.G. Cioffi, Jr. (SPON: T.L. Koppenheffer)
US Army Institute of Surgical Research, Bldg. 2657, Fort Sam Houston, TX, 78234-5012

One of the first nonspecific immune responses to thermal injury is peripheral activation of polymorphonuclear cells (PMN). We serially measured hydrogen peroxide (H_2O_2) release from PMN in 24 thermally injured patients (28-97% total body surface burns) and in 22 controls. Heparinized whole blood was hypotonically lysed and resuspended to $1x10^6$ cells/ml in Dulbecco's phosphate buffered saline (PBS). H_2O_2 was measured by horseradish peroxidase-dependent conversion of phenol red into a compound with increased absorbance. Samples were stimulated with fMLP or PMA for 30 min at 37°C. Unstimulated patient granulocytes released less H_2O_2 than those from controls. Stimulation with PMA or fMLP increased H_2O_2 release in both groups, although patient granulocytes released significantly less H_2O_2 following stimulation than controls. These data suggest that even though granulocytes from patients with thermal injury may be activated in vivo, their release of H_2O_2 is impaired.

13. Thomas, Y. et al.: Modulation of human neutrophil activation by "electronic" phorbol myristate acetate (PMA). FASEB Journal 10: A1479, 1996

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YolOne Thomas, Hedi Litime and Jacques Benveniste. CNRS URA 1442, 60206 Compiègne and Digital Biology Laboratory, 32 rue des Carnets, 92140 Clamart, France.

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2761

DIGITAL RECORDING/TRANSMISSION OF THE CHOLINERGIC SIGNAL.

J. Benveniste, P. Jurgens and J. Assa. INSERM U 200 and Digital Biology Laboratory, 32 rue des Carnets, 92140 Clamart, France.

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2762

EXPRESSION AND CHARACTERIZATION OF A RECOMBINANT MAJOR SALIVARY PROTEIN OF THE MOSQUITO *Ae. aegypti*.

W.Z. Xu, F.E.R. Simons, Z. Peng. Univ. of Manitoba, Winnipeg, MB. Canada.

Salivary proteins are responsible for allergic reactions to mosquito bites. Recombinant salivary proteins will facilitate diagnosis and immunotherapy of mosquito allergy. The gene (D7) encoding the 37kDa major salivary protein of *Ae. aegypti* was cloned and expressed by Dr. A. James. We used a new baculovirus system to produce the recombinant D7 protein. The D7 cDNA* was inserted into the baculovirus expression vector pBlueBacHis A, following in frame the sequence which encodes a tract of six histidine residues, allowing a simple metal affinity purification of the recombinant proteins. Sf9 cells were co-transfected with purified pBlueBacHisA/D7 plasmid and linear AcMNPV DNA. Ten strains of the recombinant virus were plaque-cloned. These recombinants were identified using PCR. Employing Western blot with rabbit anti-D7 protein*, we proved that all of these recombinant viruses expressed D7 protein in both infected Sf9 cells and culture supernatants. Two bands were found in the Western blot; a 42kDa band which represented the D7 fusion protein and a 37kDa band. The two bands also bound to human IgE and IgG in Western blot using mosquito-allergic sera. The recombinant baculovirus D7-8 which showed a higher expression was chosen to prepare a virus stock. Its highest expression was given 36 hours after infection. Whether the 37kDa protein is the transcribed or post-translation modified product remains to be identified. * kindly provided by Dr. A. James.

2763

T CELL EPITOPE MAPPING WITH SHORT OR LONG SYNTHETIC PEPTIDES.

A. Kettner*, Y. Chvatchko, R. Kämmerer, N. Dufour, G. Corradin* and F. Spertini. Division of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne; *Institute of Biochemistry, 1066 Epalinges, Switzerland.

The use of synthetic peptides for allergen desensitization has raised considerable interest, but is still largely debated. To define a rational approach to peptide immunotherapy, we have delineated T cell epitopes of phospholipase A2 (PLA2), a major bee venom (BV) allergen, using either short 15mer (SP) or long synthetic (LP) overlapping peptides (1-60, 51-99, 90-134) covering the whole PLA2 molecule. We here report that among 9 BV hypersensitive patients, proliferation of CD8-depleted PBMC to SP was in general weak and even absent in 4 out of 9 patients, despite positive serum anti-PLA2 IgE. In the other 5 patients, CD8-PBMC responded to at least one, in most cases to less than 3 peptides with proliferation indices >2. In contrast, using LP, an enhanced T cell response was observed in all patients. In 7 out of 9 patients, T cell proliferated to peptide 90-134, in 4 out of 9 to peptide 51-99, but no response to peptide 1-60 was detected. All patients responded to a mixture of LP. In short term T cell lines, a highly polyclonal response could be detected using SP in some patients, but proliferation to LP was again more vigorous. Using western blots and chemiluminescence, no-significant IgE binding to LP was detected except in one patient. Taken together, T cell epitope mapping using short peptides may not allow full delineation of all potential epitopes. Our results furthermore do not support the concept of rare dominant T cell epitopes. Since they induce a significant T cell response but do not bind IgE *in vitro* or only weakly, LP might represent rational candidates for peptide immunotherapy.

2764

A NOVEL 7.6 kD ALLERGEN FROM BEE VENOM: ISOLATION AND CHARACTERIZATION.

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Division of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois, 1011 Lausanne; *Institute of Biochemistry, 1066 Epalinges; **Department of Medical Biology, Centre Médical Universitaire, 1211 Geneva, Switzerland.

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The 7.6 kD protein appears to be a novel BV allergen, generating an IgE response in a high proportion of patients. Its clinical relevance and interest in a synthetic allergy vaccine will have to be defined further.

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8. Benveniste, J. et al.: A simple and fast method for in vivo demonstration
of electromagnetic molecular signalling (EMS) via high dilution or computer
recording. FASEB Journal 13: A163, 1999

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162.7

THE EFFECTS OF JP-8 JET FUEL ON ATP CONCENTRATION IN AGAR-FILLED PRECISION CUT RAT LUNG SLICES IN DYNAMIC ORGAN CULTURE.

A. Hays, J. Wijeweera, M. Witten and R.C. Lantz. University of Arizona, Tucson, AZ 85724

Agar-filled adult rat lung explants were cored and precision cut, using the Brendel/Vitron tissue slicer, in oxygenated and ice-cold Krebs-Bicarbonate slicing buffer. Slices were cultured on stainless steel screens located as half-cylinders in cylindrical Teflon cradles. The cradles were loaded into standard scintillation vials containing Waymouth's medium plus 10% fetal calf serum (1.7ml) and different concentrations of JP-8 jet fuel. Concentrations of jet fuel were 0.5 mg/ml, 1.0 mg/ml, and 1.5 mg/ml in medium. The incubator allowed for rotation of the Teflon cradles inside the vials thus exposing the slices to alternating gaseous and liquid phases that improved oxygen concentration in the tissue. Slice viability was assessed at 2, 4, and 6 hours using ATP content. There was decrease in ATP concentration concomitant with the increase in JP-8 jet fuel. A tissue slice, as opposed to cell culture, models interactions between heterogeneous cell types comprising the lung parenchyma and thus provides a controlled system for the study of pulmonary toxicology in vitro. Supported in part by AFOSR F49620-97-1-0217-P00001 and DOD AASERT Program.

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LONG TERM EFFECT OF CIGARETTE SMOKED-INDUCED RESPIRATORY CHANGES WITH HIGH PROTEIN NUTRITION.

R. Maruyama, Y. Ishihara, J. Kagawa and Y. Fukuda
Miyagi Univ., Sendai, Tokyo Women's Univ. Tokyo, Chiba Univ. Chiba Japan

Previous studies have demonstrate that low protein facilitates cigarette smoke induced ventilatory stimulation without morphological changes in broncho-pulmonary architecture.The present study was long term effect of cigarette smoking with high protein nutrition on physiological function of respiratory system, we examined ventilatory parameters of mice exposed to cigarette smoke and fed with high protein diet using whole-body plethysmograph.

BDF1 (female, 5weeks old) mice were exposed to non filter cigarette smoke in exposing chamber(0.04m³) on 5days/week for 7months and were simultaneously given high protein (130% of normal) diet. One cigarette contained 28mg tar and 2.7mg nicotine. Other cigarette smoke exposed mice were given normal protein diet. Following a total 7months exposure to cigarette smoke with or without high protein nutrition. Tidal volume (V_T) , respiratory frequency (f) and minute ventilation ($\dot{V}_E$) were measured by precision whole-body plethysmograph in unretained-awake condition. Control mice were inhalated clean air and were fed by normal diet.

The body weight gain was inhibited in cigarette smoke exposed with high protein group. Furthermore, f and $\dot{V}_E$ were increased by smoking in the high protein nutrition. However, respiratory stimulation was suppressed by the high protein ingestion.

We suggest that high protein nutrition inhibited cigarette smoke induced ventilatory changes.

162.9

A SIMPLE AND FAST METHOD FOR IN VIVO DEMONSTRATION OF ELECTROMAGNETIC MOLECULAR SIGNALING (EMS) VIA HIGH DILUTION OR COMPUTER RECORDING.

J. Benveniste, J. Aïssa and D. Guillonnet. Digital Biology Laboratory, 92140 Clamart, France.

For 15 years, we have observed specific biological activity in highly dilute solutions [1,2]. Transferring to water an EMS via electronic circuitry or digital recording shows that EMS is composed of Hertzian frequencies < 20 kHz [3-4]. We now present a simple and fast method to reveal EMS in water. Acetylcholine (ACh), bradykinin (BK) or histamine (H) induce blueing in the skin of guinea pigs and rabbits preinjected IV with Evans blue. ACh, and acetate-choline (AC) as control, both 1 mM, were diluted in water 3 times 1000-fold to 1 pM either with gentle mixing (Mx) or vortexing for 15 sec (V). Blue spots resulted from intradermal injection: AC 1 pM V, 1.3 ± 0.8 (diameter in mm, mean ± 1 SD), n=9; ACh 1 pM Mx, 1.6 ± 0.8, n=11; same V, 12.9 ± 4.9, n=17, $p<0.05$; ACh 1μM, 18.3 ± 4.7, n=11. ACh 1 pM V and 1 μM were inhibited by atropine. Similar results were obtained with BK and H, 1 pM or highly dilute (-18 to -30 M), or with digitized signals applied to water (not shown). Thus violent agitation endows full activity to a solution in which the number of physical molecules is too low to otherwise induce an effect. This activity is presumably carried by "informed" water (this meeting), that is, polarized water dipoles associated with charged molecules [5]. [1] Davenas et al., Eur. J. Pharmacol., 1987, 135:313; [2] Davenas et al., Nature, 1988, 333:816; [3] Aïssa et al., J. Immunol., 1993, 150:A146; [4] Benveniste et al., J. Allergy Clin. Immunol., 1997, 99:S175; [5] Del Giudice et al., J. Biol. Physics, 1994, 20:105. Supported by DigiBio S.A.

162.10

THE MOLECULAR SIGNAL IS NOT FUNCTIONAL IN THE ABSENCE OF "INFORMED" WATER.

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Water is able to "store" electromagnetic molecular signals (EMS) from high dilutions, electronic circuitry or computer recording [1-3]. We thus studied the role of water in molecular function at low and high concentration: (a) Acetylcholine (ACh) diluted with gentle mixing (Mx)to 1 pM caused 3.2 ± 0.5, n=9 (%, mean ± 1SD) coronary flow changes (CFC) in isolated guinea-pig hearts. The same dilution vortexed for 15 sec (V) induced 22.6 ± 1.1 CFC, n=14 compared to ACh 1 μM V, 31.0 ± 1.9, n=6, both inhibited by atropine. Similar results on CFC were obtained with ionophore A23187 (Io) and by skin testing (this meeting). (b) ACh 1 μM Mx induced CFC of 16.7, n=1; same V, 29.5 ± 5.1, n=4 ; Io 1μM Mx, 12.6 ± 1.6, n=3; same V, 18.8 ± 2.1, n=4. (c) ACh or Io 1μM, in 100% ethanol V (E100V), in 80 ethanol/20 water Mx (E80Mx), or in E80V, were digitally recorded and replayed to isolated hearts. Results on CFC were: ACh E100V, 4.3 ± 1.0, n=3; ACh E80Mx, 8.0 ± 0.6, n=3; ACh E80V, 22.7 ± 2.3, n=3, $p<0.05$; Io E100V, 3.3 ± 0.7, n=3; Io E80V, 18.3 ± 1.6, n=3, $p<0.05$. Violent agitation appears to "inform" water with EMS stemming from molecules, seemingly too weak to support the biological response. The key role of water as the acting factor - whose physical basis remains unclear - is typified by the data in (c): agonists in effective concentration do not emit enough specific EMS unless vortexed in water. [1] Davenas et al., Nature, 1988, 333:816; [2] Aïssa et al., J. Immunol., 1993, 150:A146; [3] Benveniste et al., J. Allergy Clin. Immunol., 1997, 99:S175. Supported by DigiBio S.A.

CONTROL OF BREATHING: CHEMOREFLEXES (163.1-163.2)

163.1

RHYTHMIC DISCHARGE OF LOCUS COERULEUS NEURONES IN LOW CALCIUM/HIGH MAGNESIUM: CHEMOSENSITIVITY AND GAP JUNCTIONS.

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In the *in vitro* brainstem-spinal cord (neonatal rat) the discharge of chemosensitive locus coeruleus (LC) neurones is synaptically modulated at respiratory frequency (Oyamada et al., *J. Physiol. (Lond.)* In Press). In the present experiments lowering the Ca^{2+} concentration (2.0 to 0.2 mM) and increasing the Mg^{2+} concentration (1.5 to 5.0 or 10 mM) suppressed respiratory activity and, in whole cell recordings, generated a stable rhythm of subthreshold oscillations (SRO) of membrane potential (mean frequency, 0.2 Hz) with superimposed bursts of spikes in all LC neurones (n = 18). Increasing the bath CO_2 concentration (2 to 8%, pH 7.8 to 7.2) resulted in a 2-3 mV depolarization and increase in SRO frequency (mean 39%, n = 7 of 9). Bath addition of TTX (0.2-1.0 μM) suppressed both spikes and the SRO (n = 7), but not the depolarization at 8% CO_2 (n = 2). QX-314 (2-4 mM) in the pipette suppressed spikes but not the SRO (n = 6). Bath addition of carbenoxalone (300 μM) suppressed the SRO, but did not reduce the amplitude of TTX-sensitive spikes. We conclude: that the SRO is dependent on transmission across intact gap junctions; that this transmission is not mediated by Na^+ spikes but does involve an active Na^+ conductance; and that in neonatal LC neurones gap junction transmission is not blocked by levels of acidosis which increase the excitability of LC neurones and increase respiratory output. Supported by DFG Sche 46/12-2.

163.2

SIMULTANEOUS MEASUREMENT OF INTRACELLULAR pH AND MEMBRANE POTENTIAL IN LOCUS COERULEUS NEURONS DURING HYPERCAPNIA.

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We simultaneously measured intracellular pH (pH_i) and membrane potential in Locus Coeruleus (LC) neurons (the LC is hypothesized to be a region which contains a high percentage of central chemoreceptors) to correlate their excitability with a decrease in pH_i. Recordings were made with microelectrodes (125-150MΩ) that contained 3 M K-acetate and the pH-sensitive fluorescent dye, pyranine (10 mM), which was iontophoresed into the neurons. During hypercapnic acidosis, we found that 5 of 6 LC neurons acidified and maintained that acidification. All 5 neurons depolarized (subsequent to intracellular acidification) during hypercapnic acidosis. Of those 5 neurons, 3 neurons were spontaneously active and had an increased firing rate (again, subsequent to intracellular acidification) during hypercapnic acidosis. Also, of those 5 neurons, 3 neurons had an increased input resistance (R_N). The other 2 neurons had no change in R_N. In contrast, 1 of 6 LC neurons acidified initially and exhibited pH_i recovery during hypercapnic acidosis. Subsequent to, and during the acidification and pH_i recovery, the neuron hyperpolarized and had a decrease in R_N. In summary, we show that during hypercapnic acidosis, the majority of LC neurons acidify and maintain that acidification, and have an increase in excitability. This work supported by NIH Grant R01-HL56683.

9. Benveniste, J. et al.: Specific remote detection of bacteria using an electronic/digital procedure. FASEB Journal 13: A852, 1999

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ABSTRACTS
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645.17

COMMUNITY ACQUIRED PNEUMONIA DUE TO *MYCOPLASMA PNEUMONIAE* PRESENTING WITH TRANSIENT MASSIVE PROTEINURIA. P. Dileep Kumar and Dorothy Bradford (SPON: Burton C. West). Dept of Medicine, Huron Hospital, Cleveland, OH 44112.

A 23 y/o man presented with throat pain & cough for 2 wks. He later developed left chest pain & fever. Exam: RR 32, exudative pharyngitis, dullness & ↓ breath sounds over left chest. PO_2 52 mm Hg on room air. WBC 20,200/μL (N 70%, bands 20%, L 3%, M 7%), creatinine 0.8 mg/dL. Urine: protein of >300 mg/dL, 3-5 WBC & 3-5 RBC/hpf & granular casts. A 24-hour urine protein was 2349 mg. CXR: LLL infiltrate, sputum culture: normal respiratory flora with group A β-hemolytic streptococci. Rapid strep test on throat swab: positive. *M. pneumoniae* antibody (ELISA) IgG was zero and IgM was 1.121 U/mL (< 0.77). He was treated with doxycycline for 2 wks with complete recovery. *M. pneumoniae* titre after 4 weeks: IgG 0, IgM 1.470 U/mL. Proteinuria at 3 weeks: 94 mg/24 hours. Our interpretation was that the sputum GABS was from the pharyngitis and the high titres established *M. pneumoniae* as the cause of the pneumonia.

Renal involvement with massive proteinuria is unusual in *M. pneumoniae* pneumonia. Direct infection of the kidneys or immunological mechanisms may be responsible for this manifestation in our patient.

645.18

GENE REPLACEMENT AT THE HEMOGLOBIN RECEPTOR LOCUS IN *NEISSERIA MENINGITIDIS*

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The ability of bacterial pathogens to establish infections is in part due to the ability of the organism to scavenge essential nutrients, such as iron, from human tissues. We studied the genetic locus in *N. meningitidis* containing the human hemoglobin receptor gene, *hmbR*. *hmbR* was located between a homolog of the iron-regulated gene, *pigA*, from *P. aeruginosa*, and *col*, which encodes a protein with 63% identity to an *E. coli* collagenase. However, some strains did not react with *hmbR* specific primers and Southern blots confirmed that these strains did not contain *hmbR*. The nucleotide sequence of the region between *pigA* and *col* in a serogroup Y strain showed that *hmbR* was eliminated from the genome and replaced by a 1.1 kb sequence containing an 809 bp novel ORF, *orfB*. OrfB contained a 94 aa motif which was 35% identical to a region of the neisserial transferrin binding protein, TbpB. The intergenic space between *pigA* and *col* in a W-135 strain contained a second novel 1368 bp ORF, *orfC1*. Although *orfB* and *orfC1* did not have nucleotide identity, the predicted aa sequences from these ORFs had 55% identity. Southern hybridizations and PCR analysis of the *pigA-col* locus in other isolates confirmed that *hmbR*, *orfB* or *orfC* were mutually exclusive. *hmbR* was in 100% of serogroup B, 96% of serogroup C strains, and 68% of serogroup A isolates. In contrast, 90% of serogroup Y isolates carried *orfB* and only 7% of this group carried *hmbR*. Other isolates of serogroup A meningococci contained *orfC* alleles. The meningococcal *hmbB* locus is characterized by an unusual event in which *hmbR* has been exchanged with novel genes which may affect the ability of this pathogen to utilize iron.

645.19

INFECTION OF ENDOTHELIAL CELLS BY *M. leprae*.

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The unique predilection of *M. leprae* for peripheral nerves is a central event in the pathogenesis of Hansen's Disease, but the mechanisms responsible for this are unknown. In experimental lepromatous neuritis in the armadillo, *M. leprae* colonizes endothelial cells of epineural blood vessels even in sites of minimal infection, suggesting that interaction between these cells and *M. leprae* may play an important role in the selective localization of this organism to peripheral nerve. To study the mechanisms involved, we have begun to examine the interaction between *M. leprae* and human umbilical vein endothelial cells (HUVEC) *in vitro* using light microscopy, scanning and transmission electron microscopy, and confocal laser scanning microscopy. When *M. leprae* were added to confluent monolayers of HUVEC, uptake increased slowly to a maximum at 24 hr. Maximal percentages of infected cells were similar at ratios of organisms:HUVEC over a range of 25:1 - 100:1. *M. leprae* were found within phagocytic vacuoles at all time points. The kinetics of ingestion of *M. leprae* by HUVEC differ from that previously observed in macrophages. HUVEC appear to offer a suitable model *in vitro* for the study of selective mechanisms of interaction between *M. leprae* and endothelial cells.

645.20

The effect of Staph. aureus on TNFa and IFNg levels during wound healing in a canine open fracture model

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Background: Cytokines play a critical role in wound healing in the host immune response and the effects of bacterial contamination on pro-inflammatory cytokine levels are not known in open fracture wound fluid. Methods: Open fractures, created in ten adult mongrel dogs, were characterized by bone loss and soft tissue injury and were repaired with intramedullary nails. Staph. aureus (1,000,000 CFU) was injected at the fracture site (n=4). Microdialysis probes [MW cutoff of 100kD] were implanted at the fracture site and in a contra-lateral limb for 7 d. Cytokine and glucose levels, as a receovery standard, were determined by immunoaffinity capillary electrophoresis and a commercial kit, respectively. Results: Fracture site TNFa levels at day 0 (196 + 91) increased on days 1-6 (5831 + 3714, $p<0.02$) and then decreased on day 7 (1985 + 882). No effect on fracture site TNFa levels was observed with staph. Aureus. Fracture site IFNg levels at day 0 (242 + 158) increased on days 1-6 (4097 + 2300, $p<0.05$) and decreased on day 7 (1476 + 1341). IFNg levels at the fracture were significantly decreased at days 1-6 (2171 + 1612, $p<.05$) compared to non-inoculated fractures site. Significance: Our data suggest that at the fracture site IFNg levels but not TNFa levels are modulated by staph. aureus in an open fracture.

645.21

FREQUENCY OF APOLIPOPROTEIN E (APOE) ALLELE TYPES IN PATIENTS WITH *CHLAMYDIA*-ASSOCIATED ARTHRITIS AND OTHER ARTHRITIDES.

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Genetic background is important in determining whether certain infecting bacteria disseminate to the joint and cause arthritis. We assessed whether APOE genotype is associated with presence of DNA from Chlamydia or other bacteria in synovial tissues of patients with various arthritides. Nucleic acids from synovial tissues of 135 patients were screened by PCR for DNA from *Chlamydia trachomatis*, *C. pneumoniae*, and other bacteria (pan-bacteria). *APOE* genotype was determined by a PCR-based method for all patients in each of 4 resulting groups comprised of about 35 individuals each, positive for *C. trachomatis* only, *C. pneumoniae* only, other bacteria, or no bacteria. RT-PCR was used to assess synovial *APOE* expression. The latter assays confirmed that APOE mRNA is present in synovial tissue. Determination of *APOE* genotype showed that patients PCR-negative in all assays, and those positive in the *C. trachomatis*- and pan-bacteria- (excludes *Chlamydia*) directed assays, had distributions of the *APOE* 2, 3, and 4 alleles mirroring those of the general population (i.e., 8%, 79%, 13%, respectively). In contrast, 68% of patients with *C. pneumoniae* DNA in synovium possessed a copy of the 4 allele. These results indicate that no association exists between *APOE* genotype and synovial presence of *C. trachomatis* or other bacteria. However, individuals bearing at least one copy of the *APOE* 4 allele may be at increased risk for synovial infection by *C. pneumoniae*. (NIH grant AR-42541).

645.22

REMOTE DETECTION OF BACTERIA USING AN ELECTROMAGNETIC/ DIGITAL PROCEDURE.

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Previous studies suggest that the electromagnetic molecular signal (EMS) can be digitally recorded and replayed [1-4]. We electronically captured, digitized, and transmitted the specific EMS of bacteria to a biological system sensitive to this EMS. First we digitally recorded EMS from *E.coli K1, staphylococcus* (3.5 million/ml) or saline. Latex particles, sensitized by an *E.coli K1* antibody (ab), aggregate to *E.coli K1* (kit, Pasteur Diagnostics). We then set up the reagent concentrations so as to obtain minimal aggregation. Next, we applied for 2 min the recorded EMS to ab-latex and ag which we subsequently mixed. After allowing the mixture to migrate for 13 min at 37°C in a capillary, we examined it under a microscope. A CCD camera generated 3 pictures that were digitally analyzed. *E.coli K1* EMS induced the formation of larger aggregates than that of *Staphylococcus* or saline. The magnitude of aggregation varied from day to day. Yet, by comparing *E.coli* data from controls of the same day, we seldom failed to detect *E.coli* in hundreds of experiments (mean of differences: 150%). Thus, we are clearly able to identify a bacteria by playing its EMS signal. The latter is specific, since the effect of *staphylococcus* EMS on *E.coli* ag/ab reaction was close to that of saline. Since EMS travel a long distance in the form of a ".wav" file, it should become possible to detect any immunogenic substance from a remote location. [1] Davenas et al., Eur. J. Pharmacol., 1987, 135:313; [2] Davenas et al., Nature, 1988, 333:816; [3] Aïssa et al., J. Immunol., 1993, 150:A146; [4] Benveniste et al., J. Allergy Clin. Immunol., 1997, 99:S175. Supported by DigiBio S.A.