7. Benveniste, J. et al.: Digital biology: Specificity of the digitized molecular signal. FASEB Journal 12: A412, 1998

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ABSTRACTS

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2392

DIGITAL BIOLOGY: SPECIFICITY OF THE DIGITIZED MOLECULAR SIGNAL. J. Benveniste, J. Aïssa and D. Guillonnet, . Digital Biology Laboratory, 32 rue des Carnets, 92140 Clamart, France.

For several years, we have been able to activate various biological systems using the electromagnetic (EM) signal of the agonist instead of the molecule itself. Signals can be applied electronically, in real time, to target cells or organs, or digitally recorded on a multimedia computer, either directly using a purpose-designed transducer or via the telecommunication network, and then replayed. Here, we investigated the specificity of digitized EM signals of acetylcholine (dACh) and histamine (dH). The latter or, as controls, similarly recorded water (dW) or white noise (dWN) were applied to isolated perfused guinea-pig hearts. Coronary flow was monitored for 30 min. Variations - as mean % $\pm$ 1SD [nb of measures] - were: dW, 5.9 $\pm$ 2.0 [9]; dWN, 6.0 $\pm$ 1.7 [17]; dACh, 21.5 $\pm$ 6.9 [10]; dH, 15.0 $\pm$ 1.2 [4]; Ach (0.1 μ, as positive control, 21.3 $\pm$ 11.6 [4]. In other experiments, hearts were perfused with ACh or H1 receptor blockers at 1 μM. Under atropine, variations were: dACh, 8.0 $\pm$ 3.5 [6]; ACh (0.1 μM), 12.5; under mepyramine: dH, 5.1 and 4.8; H (0.1 μM), 5.0. After washout of atropine, dACh activity was restored to 23.7 $\pm$ 3.3 [3]. In short, signals were as active as original molecules and atropine inhibited both ACh and dACh while mepyramine inhibited both H and dH. Thus, EM "ligands" act on specific receptors, closely mimicking original molecules. These results, together with our previous findings, suggest that molecules emit specific hertzian waves than can be digitized, modified, transferred and replayed. They open the way to digital biology and medicine. *Supported in part by Association Science Innovante and DigiBio SA.*

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TUMOR NECROSIS FACTOR ALPHA (TNF-α) IN HOSPITALIZED PATIENTS WITH CONGESTIVE HEART FAILURE. P. Somasundaram, B.H. Sung, C. Vavilala, A. Luzier, J.M. Hyatt, M.F. Wilson. Millard Fillmore Health System and SUNY, Buffalo, NY 14209

TNF-α has been reported to be elevated in patients with congestive heart failure (CHF) but the relationship between TNF-α level and acute heart failure is not known. Thus, we examined whether TNF-α level is elevated in CHF patients with acute cardiogenic pulmonary edema. Blood samples for TNF-α level were taken from 14 patients within 24 hours of admission and the time of discharge after treatment. Patients who had signs of infection were excluded. Blood samples were analyzed with high sensitivity kit. Mean age of the group was 72 years and left ventricular ejection fraction was 34%. 12 of 14 patients were NYHA class 3 or 4. The length of hospital stay ranged from 2 to 14 days (mean =5.5 days). TNF-α level at admission was significantly higher than the time of discharge (5.5 vs 4.2 pg/ml, $p < 0.02$). Multiple regression analysis showed that the duration of hospitalization ($r=0.80$, $p< 0.003$) and TNF-α level at admission ($r=0.71$, $p < 0.02$) were significant predictors of change in TNF-α level. **Conclusion**: TNF-α level is elevated in CHF patients who have acute exacerbation. Treatment significantly reduce TNF-α levels suggest TNF-α may be related with acute symptoms. The greater change in TNF-α level was associated with the duration of hospitalization.

MUSCLE PLASTICITY (2394-2397)

2394

He-Ne LASER ACTION IN THE REGENERATION OF THE *TIBIALIS ANTERIOR* MUSCLE OF MICE. Amaral A.C., Salvini T.F., Parizzoto N.A. Laboratório de Neurociências, PPG-CFS, UFSCar-SP

Objective: The aim of this work was to analyze the effect of different doses of He-Ne laser in the regeneration process of the *tibialis anterior* muscle of mice. Material, Methods and Results: Fifteen male mice received a single muscular injection (0.5 mg/Kg) of myotoxin ACL (*Agkistrodon contortrix laticinctus*) in the middle region of the *tibialis anterior* muscle. The injury was induced in both, right and left hindlimb, with the right leg submitted to the laser treatment and the left leg used as a control. The animals were subdivided in three groups, distinguished by the dose of laser used in the treatment, 2.6, 8.4 and 25 J/cm2 respectively. The treatment was based on five consecutive days of application. The application were performed punctually on the skin in the corresponding region to the venter of the right muscle. Similar procedure was performed on the left leg, nevertheless, with the apparatus turned off (control). After 21 days of injury the animals were sacrificed and their muscles removed for morphometrical and histological analyses. The comparative morphometrical analyses, performed inside of each group, showed significant difference ($p = 0.02$) between the areas of the muscle fibers only for the 2.6 J/cm2 group. The histological analyses showed evidence of a greater concentration of mitochondria in the treated muscles related to the control, also exclusive of the animals treated with the dose of 2.6 J/cm2. Conclusion: The results suggest that only He-Ne laser of 2.6 J/cm2 was efficient to promove an improvement in the quality of the regenerative process of the skeletal muscle tissue of mice. Financial Support: CAPES, FAPESP

2395

Ca^{2+} SIGNALS MEDIATING CYTOCHROME C TRANSACTIVATION IN SKELETAL MUSCLE. M. Di Carlo, D. Freyssenet, and D.A. Hood. Depts. of Kinesiology and Biology, York University., Toronto, Ontario, M3J 1P3, Canada.

We have previously demonstrated that cytochrome c transcription is upregulated by an A23187-induced increase in cytosolic Ca^{2+} concentration. The increased expression of cytochrome c appears to involve a protein kinase C (PKC) pathway. To evaluate which PKC isoform is involved, we co-transfected L6E9 muscle cells with a -326 cytochrome c promoter-CAT construct, as well as calcium-sensitive (α and β_{II}) or insensitive (δ) PKC isoform expression vectors. The results demonstrated a 2.8- and 2-fold enhancement of the A23187 effect for PKC α and PKC β_{II}, respectively. The Ca^{2+}-insensitive PKC δ isoform was without effect. To determine if mitogen-activated protein kinase (MAPK) was acting downstream of PKC, we examined the time course of MAPK activation in response to A23187 treatment using a phospho-specific MAPK antibody. MAPK activation increased after 1 hour, was maximal at 2 hours and declined to approximately 50% of maximum by 4 hours. Pretreatment of the cells with PD98059, a MEK inhibitor, attenuated the A23187 effect on cytochrome c transactivation by 37% (n=5). These data suggest that Ca^{2+}-mediated increases in cytochrome c transactivation in muscle cells are due, in part, to the activation of a PKC-MAPK pathway. (Supported by NSERC, Canada)

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THE EFFECT OF RECURRENT CONTUSION IN THE *TIBIALIS ANTERIOR* MUSCLE – MORPHOLOGICAL AND HISTOCHEMICAL ANALYZES. Minamoto, V. B., Bunho, S. R., Salvini, T. F. Laboratório de Neurociências, PPG-CFS, Universidade Federal de São Carlos, S.P.

Introduction: Skeletal muscle has the ability to adaptation of different kinds of stimulus, such as hormones alterations, physical training and muscle activity. This adaptation can be also noted in its morphological and histochemical characteristics. The aim of this work was to analyze the effect of chronic contusion in the tibialis anterior muscle of rats. Material and Methods: Right *tibialis anterior* of Wistar rats (n=8)) were submitted at 8 consecutive contusion, once a week, during 8 weeks. The animals were immobilized in lateral position and the contusion was produced by a drop-mass equipment. After 30 days the animals were sacrificed and both, right and left muscles were weighted, frozen in liquid nitrogen and analyzed. The muscles were cut in serial cross-sections (10 um). The injury signs and the muscle fiber types were observed by toluidine blue staining, acid phosphatase and m-ATPase reaction. Results: The control muscles were heavier than the injured muscles ($p= 0.03$, student-T, test). The damaged muscles showed a great incidence of signs of lesions, mainly split fibers and muscle fibers with centralized nucleus. These signs were observed in the deep region of all muscles. The right muscles showed a higher incidence ($p= 0.04$) of the type IIc fiber, when compared with the left muscles. Conclusion: The decrease of weight observed in the injured muscles can be the result of protein depletion, resulting from the chronic injury. The recurrent contusions can also change the incidence of type fiber, probably because the lesions. Financial Support: FAPESP and Capes

2397

IMPAIRED UTILIZATION OF EXOGENOUS SUBSTRATES BY RAT SKELETAL MUSCLE AFTER HINDLIMB SUSPENSION. B. Lujan, F. Garcia-Ghinis and L.A. Bertocci. Presbyterian Hospital of Dallas IEEM and UT Southwestern Medical Center, Dallas, TX.

Skeletal muscle disuse atrophy reduces oxidative enzyme activity, although increased glycogenolysis and lactate accumulation is not always seen. An explanation was that atrophy was so severe that enzyme activity per unit muscle mass is not reduced. To test this, we perfused the isolated rat hindquarter with a mixture of [3-^{13}C]lactate+pyruvate, [1,3-^{13}C]acetoacetate+β-hydroxybutyrate and [U-^{13}C]fatty acids and used ^{13}C NMR isotopomer analysis to determine relative acetyl-CoA use in rested versus contracted (30 minutes in situ unilateral contraction) muscle in control vs 28 day hindlimb suspended rats (n=8 each). In control muscle, contraction increased uptake of exogenous ([3-^{13}C]lactate+pyruvate, Fc2 and [U-^{13}C]fatty acids, Fc3) versus endogenous (unlabeled, Fc0) acetyl-CoA (* $p<0.05$ vs rested control). In contrast, although Fc2 was higher in rested suspended muscle, it did not increase with contraction. We conclude that atrophied muscle is more dependent on carbohydrate oxidation during rest but atrophy prevents normal increases in exogenous carbohydrate use during contraction.

		CONTROL		HINDLIMB SUSPENDED	
	acetyl-CoA	RESTED	CONTRACTED	RESTED	CONTRACTED
Fc0	unlabeled	0.97±0.02	*0.67±0.08	*0.89±0.03	0.80±0.06
Fc2	[2-^{13}C]-	0.03±0.02	*0.26±0.07	*0.11±0.03	0.20±0.06
Fc3	[1,2-^{13}C]-	0.00±0.00	*0.07±0.03	0.00±0.00	0.00±0.00

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INTRODUCTION

For several years, we have activated various biological systems using the electromagnetic (EM) signal of the agonist molecule instead of the molecule itself. Signals are applied electronically, in real time, to target cells or organs. Alternatively, they are digitally recorded on a computer, either directly, using a purpose-designed transducer, or via the public switched network, and then replayed [1-9]. One of the main questions in this complex process of detection, recording, transfer and replay, is the integrity of the applied signal, which is a prerequisite for the specific expression of the molecular signal.

We therefore investigated the specificity of digitized EM signals of acetylcholine (d-ACh) and histamine (d-H).

MATERIALS AND METHODS

Heart preparation

Isolated hearts from male Hartley guinea-pigs, ~= 300 g, were perfused according to the Langendorff method using Krebs-Henseleit buffer (pH 7.4) gassed with O2/CO2, 95/5 %, at a pressure of 40 cm H2O at 37°C.

Recording

ACh, or sodium acetate + choline chloride (AC) or H at 1µM, or water (W), were recorded (6 sec, 16 bits, 44 kHz) using a purpose- designed transducer and a computer equipped with a sound card.

Assays

d-ACh and d-H were applied to isolated guinea-pig hearts, perfused or not with the ACh inhibitor atropine or the H1 receptor blocker, mepyramine, both at 1 µM. d-W or d-AC, and ACh or H (1 µM), were also applied as negative and positive controls respectively. Coronary flow was then measured every min for 30 min and changes were calculated as % maximal coronary flow variation compared to three "time 0" values. Student's t-test for unpaired variates (Plot 40, Sigma Plot) was used to assess statistical significance.

Mechanical parameters (min. and max. tension, heart rate) were observed and recorded using dedicated software.

RESULTS

The effects on the maximal variation in coronary flow are shown in Table 1. ACh, H (1 µM), d-ACh, d-H induced highly significant changes, whereas d-W or d-AC were indistinguishable from spontaneous flow variations.

Atropine inhibited both the effects of ACh and d-ACh, but not those of H and d-H. Mepyramine inhibited the effect of both H and d-H but not those of d-ACh.

Variations in coronary flow following application of d-ACh and d-H were highly significant compared with controls, d-W and d-AC.

A typical effect of d-ACh on heart frequency and its inhibition by atropine are shown (figure 1).

[Vasodilation was induced in guinea-pig skin by d-ACh, whereas d-AC was ineffective (figure 2).]

Table 1

Effect of the anticholinergic atropine or the anti-H1mepyramine on the coronary flow variation, in % ± 1 SD (nb of experiments), induced by d-ACh or d-H in isolated guinea-pig hearts

	No Inhibitor	**Atropine**	**Atropine washed**	**Mepyramine**
d-ACh	19.5 ± 7.4 (a) [21]	7.3 ± 2.8(b) [10]	16.9 ± 6.9 (c) [7]	19.7 ± 3.9 (c) [3]
d-AC	3.5 ± 1.6 [8]			
ACh 1 µM	26.6 ± 8.3 [16]	8.8 ± 3.3 (d) [3]	24.6 ± 7.9 (e) [7]	
d-H	14.3 ± 2.5 (f) [14]	14.0 ± 2.1 [3]		5.8 ± 1.8 [8]
H 1 µM	21.1 ± 8.4 (f) [5]	23.6 ± 4.3 [4]		8.2 ± 2.9 [6]

d-W: 4.6 ± 2.1 (28).

(a) : $p < 0.05$ (Student's t test for impaired variates) vs d-AC
ns vs ACh 1 µM
(b) : ns vs d-AC
(c) : ns vs d-ACh (no inhibition)
(d) : $p < 0.05$ vs ACh (no inhibitor)
(e) : ns vs ACh (no inhibitor)
(f) : $p < 0.05$ vs mepyramine, ns vs atropine.

Figure 1

Effect of ACh signal on hearth rate (bpm)
Inhibition by atropine
Parameter : Frequency Channel # : 1

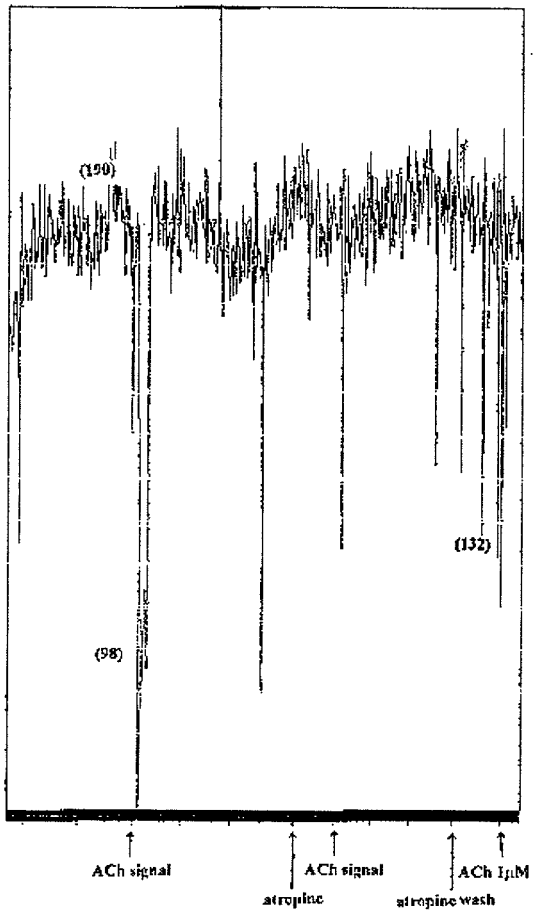

Figure 2

Skin reactions in a non-immunized guinea-pig, scanned live, following intra-dermic injection of digital acetylcholine ('computer-informed' water)

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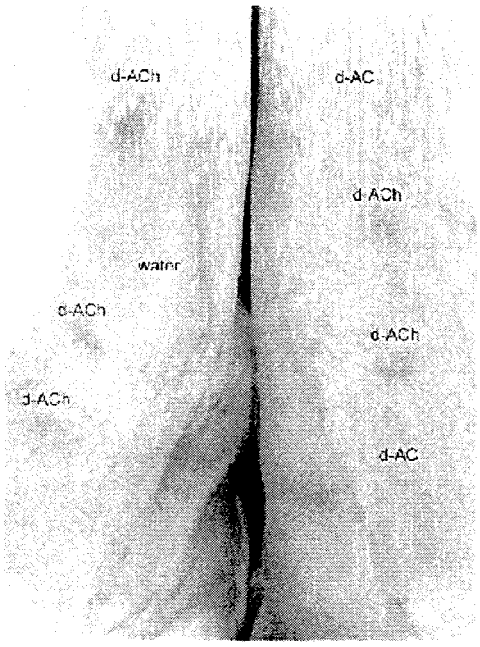

DISCUSSION

We have used a purpose-designed transducer and a sound card-equipped computer, to digitize, record and replay the signals of ACh and H to isolated perfused guinea-pig hearts.

EM signals of ACh and H influenced cardiac function in the same way as the original molecules did

The specificity of the digitized activity is evident in the fact that atropine inhibited both ACh and d-ACh, while mepyramine inhibited both H and d-H, indicating that the recorded signal interacts with the same receptor as the original molecule. These results demonstrate that at least some biologically active molecules are capable of transmitting their activity to target cells or organs in the form of electromagnetic radiation of less than 44 kHz that can be recorded, digitized and replayed.

We therefore suggest that:

1. The molecular signal consists of low frequency waveforms.

2. One of the biological roles of molecule-associated water may be to mediate this signal, a hypothesis supported by contemporary physical research [10, 11].

3. The digitized signal is specific to the originally recorded molecule. When applied to target cells or organs, it exerts the same effects as the agonist in molecular form and its effects are inhibited by the same antagonist.

CONCLUSION

These results indicate that the molecular signal is composed of waveforms in the 0-44 Khz range which are specific to each molecular entity. They open the way to purely digital procedures for the analysis, modification and transmission of molecular activity, with medical and possibly industrial applications.

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