Effect of L-arginine and its guanidino-methylated derivatives on the growth of tobacco tissue cultures

Treatment	End weight after 62 days (g)	Daily growth (mg)	Growth inhibition (%)
Control	16.38	264	0
A-10	16.22	261	0
A-100	8.49	137	49 ($P_{1\%}$)
MMA-10	9.89	159	40 ($P_{1\%}$)
MMA-100	2.32	37	87 ($P_{0.1\%}$)
DMA'-10	10.23	160	38 ($P_{1\%}$)
DMA'-100	3.84	62	77 ($P_{0.5\%}$)
DMA-10	10.62	170	36 ($P_{1\%}$)
DMA-100	3.10	44	79 ($P_{0.1\%}$)

Signs and abbreviations: A, L-arginine; MMA, N^G-monomethyl-L-arginine; DMA', N^G, N'^G-dimethyl-L-arginine; DMA, N^G, N^G-dimethyl-L-arginine; 10, 10 mg amino acid/l culture liquid; 100, 100 mg amino acid/l culture liquid.

plate containing Dowex 50×8 type ion exchange resin (Chinoin-Nagytétény, Budapest, Hungary), previously equilibrated with sodium citrate buffer (pH 3.28; 0.02 *N* Na^+). The eluting buffers used were various sodium citrate buffers[9].

As seen from the Table, at concentrations of 10 and 100 ppm MMA, DMA' and DMA respectively considerable growth inhibition could be attained after 62 days of culturing compared to the control.

After 62 days, the added L-arginine, and particularly its N^G-methylated derivatives, can be shown by ion exchange thin-layer chromatographic methods, even in alcoholic extracts of culture medium as in the tissue extracts. The 3 guanidino-methylated arginines show the following order of enrichment in the tissue compared to the control: control < arginine < MMA < DMA' < DMA. It is probable that the tobacco tissue cannot demethylate the 2 dimethyl-L-arginine. In the case of MMA the methyl group is less stable.

The data of our investigations prove that, in the case of treatment performed with guanidino-methylated arginines, a permanent inhibition of arginine incorporation, of vital importance for the tobacco tissue and its great stability, results in growth-retardion.

Zusammenfassung. Drei N^G-methylierte Derivate von L-Arginin hemmen das Wachstum der Tabak-Kallusgewebe in 10–100 ppm Konzentrationen.

E. Tyihák, M. Maróti, D. Vágujfalvi, S. Bajusz and A. Patthy

Research Institute for Medicinal Plants, P.O. Box 11 H–2011 Budakalász, (Hungary), and Eötvös Lorand University, Institute for Plantphysiology and Research Institute for Pharmaceutical Chemistry, Budapest (Hungary),
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Responses of Cerebellar Units to Stimuli Simulating Sound Source Movement and Visual Moving Stimuli

In several publications the sensitivity of neuronal network in lobuli VI and VII of the cerebellum to visual and acoustic stimuli has been reported[1–4]. Neurones, mainly Purkyně cells, were found to be responsive to clicks, tones and flashes. Recently, responses of cerebellar neurones to moving visual stimuli were described[5]. In acoustically activated neurones, a high sensitivity to binaurally presented and time shifted clicks was demonstrated, whereas a lower responsiveness to tonal stimuli was found in comparison with neurones from specific auditory nuclei[6].

As is generally assumed, the cerebellum is involved in the control of body movements[7]; and it seems probable that information about the movement of acoustic and visual stimuli is transmitted to the cerebellum. We therefore attempted to explore the responses of lobulus VI and VII neurones to stimuli, which simulate the sound source movement[8,9], and simultaneously to investigate the reaction of the same neurones to moving visual stimuli.

Methods. Experiments were performed on cats immobilized by Diplacin (muscle relaxant, with effects

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similar to D-Tubocurarine) and artificially ventilated. Pupils were dilated by atropine. All pressure points and wounds were locally anaesthetized. Either single clicks (0.5 msec duration) or trains of clicks (train frequency of 10–40 Hz, train duration 2 sec) were used for acoustic stimulation. Clicks were presented binaurally through precision condensor earphones. The sound intensity in both acoustic lines reached 40 dB SPl at the most. For identifying neurones specifically reacting to a moving sound source, trains of binaural clicks with changes of interaural delays in 50 μsec steps were used[8,9]. Such changes in ΔT are perceived as movement of the sound source from the ear to the midline and back to the ear. Experiments were performed in a sound proof chamber.

Light spots and black targets were moved by hand on a translucent tangential screen, placed 50 cm before the animal's eye. The influence of different directions of movements in the frontal plane as well as in the sagittal plane (to and from the animal) on unit activity was examined. Extracellular single unit discharges were recorded on magnetic tape and processed by a laboratory averager. Peristimulus time histograms were computed. Morphological control of the electrode localization was performed in all cases.

Results and discussion. At first neuronal activity was recorded during single click stimulation. Responses of 62 cerebellar neurones, which were excited by clicks were further analyzed. 41 neurones were tested by click trains with gradually changing interaural delay (simulating movement of the sound source). Each cell was stimulated by 10–20 different trains with different initial delay (ΔT_{max}), thus producing different velocities of sound source movement. Altogether we obtained 530 post-stimulus histograms. After thorough examination of all data, only 29 neurons proved to be stable as regards the activity during the experiment and were examined according to complete program, i.e. with all necessary control measurements.

Fifteen neurones out of the 29 (52%) reacted vigorously to trains of clicks with changing interaural delays, in comparison with the reaction, evoked by similar trains of clicks without delay. In Figure 1 responses of one such neurone are shown. Simultaneously applied clicks (without delay) weakly activated the neurone (lower part of the Figure). On the contrary, gradual reduction of the ΔT (movement of the sound source from the ear to the midline) first excites the cell, then diminishes the response to zero. Augmentation of the ΔT (sound source movement in the opposite direction) exhibits a symmetrical response pattern. Responses of this type were classified as 'symmetrical' and appeared in 6 neurones. In Figure 1 similar symmetrical response is obtained with different sequencies of stimulus time delay, i.e. in the case when clicks on the right or left ear are delayed, and also with different ΔT_{max} (500 and 300 μsec) and thus with different velocities of movement.

Figure 2 illustrates a case of more frequently found neurones – 9 units – when an 'asymmetrical' response was found. Reduction of the ΔT in the click train on the left ear (and thus the movement from right to the midline) is accompanied by a greater response, when compared with the augmentation of ΔT. A similarly preferred direction of movement for the visual stimulus was noted from right to left and downwards.

Forty-five neurones (out of the total sample of 62) were examined with moving visual stimuli. As a rule, a low responsiveness was found with a weakly expressed directional specificity. The visual receptive fields of 32 neurones were examined, in the majority of cases, however, the boundaries of receptive fields spread off the screen (more than 60 angular degrees in diameter). Wide receptive fields of the cerebellar units were characterized by a low directional specificity. 23 units (out of 45) were directionally non-specific, in directionally specific units (22) preferred direction was to one side and downwards, or to one side and upwards. Moreover, 15 units (out of 45) reacted to radial movement (to and from towards the animal head).

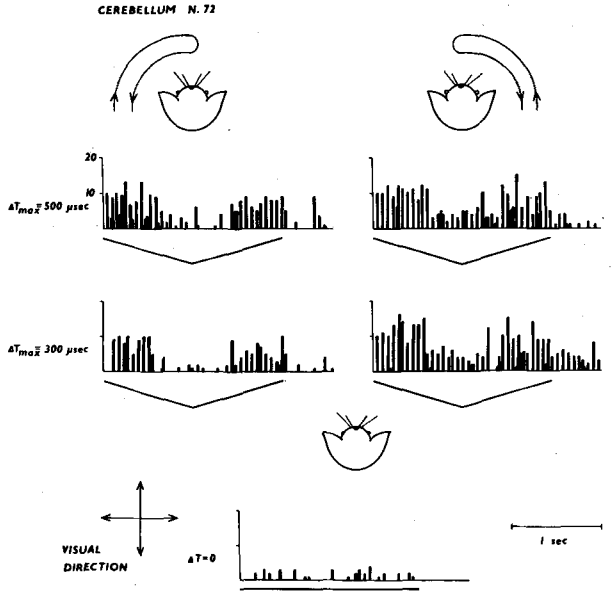

Fig. 1. 'Symmetrical' type of response of the cerebellar neuron to stimuli simulating sound source movement. Histograms below the diagram of the cat head with arrows from the left: response to gradual shortening and augmentation of ΔT with right ear stimulus delayed. Histogram below the diagram of the cat head without arrows: reaction to simultaneous binaural stimulation by train of clicks (without changing delay). Duration of the stimulation is marked by lines below the histograms, click repetition rate in the train = 20/sec. ΔT_{max} in upper histograms = 500 μsec, in histograms in the middle = 300 μsec. Poststimulus histograms computed from 20 train repetitions. Preferred direction for visual stimuli in the frontal plane marked by arrows.

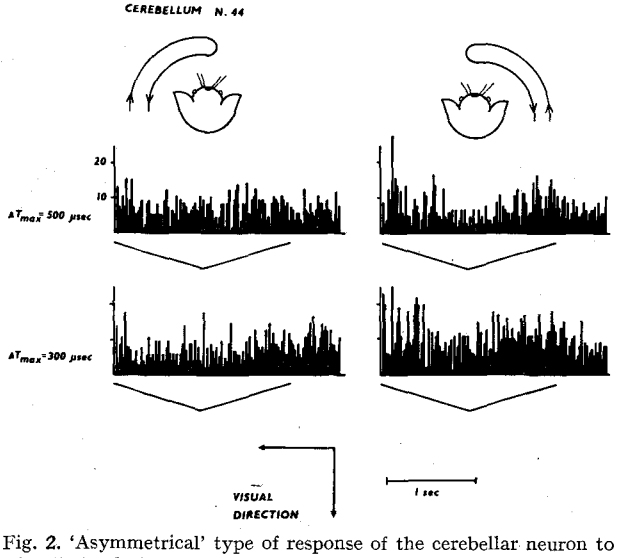

Fig. 2. 'Asymmetrical' type of response of the cerebellar neuron to stimuli simulating sound source movement. Symbols similar as in Figure 1.

Responses to both visual moving stimuli and to acoustic stimuli, simulating sound source movement, were compared in 23 neurones. Only 9 neurones exhibited responses to ΔT changing trains and responded to visual moving stimuli. Acoustical responses classified as 'asymmetrical' were characterized in 4 neurones (out of 9) by visual directional specifity and the preferred direction of movement for an acoustic stimulus agreed with the preferred direction for a visual stimulus.

It may be thus concluded that cerebellar units from area VI and VII respond to moving visual stimuli and similarly to acoustic stimuli, which simulate movement of the sound source. In comparison with neurones from specific auditory pathways[8,9], a remarkable number of neurones (31%) displayed an 'asymmetrical' type of reaction, with a certain preference for the direction of sound source movement. Moreover, 21% of neurones exhibited a 'symmetrical' type of response, i.e. without any preference for the direction of sound source movement.

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At the same time with this fact a large number of directionally non-specific visual neurones (51%) was found. Furthermore in units with preference for a certain visual direction, less expressed specificity was present, when compared with the properties of neurones from movement sensitive visual areas (e.g. the superior colliculus[10,11]). It is possible to assume from our experiments that a) the information about the movement of acoustic and visual stimuli seems to be most important for the cerebellum, b) responses to other characteristics of the stimulus (i.e. frequency, intensity, direction) are at least to some extent less expressed (e.g. [6]).

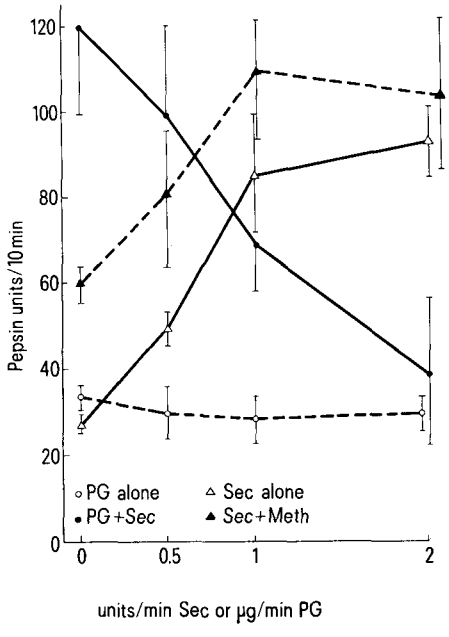
Zusammenfassung. Der Einfluss von simulierten bewegten Schall- und Lichtquellen auf einzelne Neurone im Kleinhirn wurde untersucht.

N. N. Bechtěrev, J. Syka and J. A. Altman

Pavlov Institute of Physiology, Academy of Sciences, Leningrad (USSR) and Institute of Physiology, Czechoslovak Academy of Sciences, Budejovická 1083, Praha 4 (Czechoslovakia), 16 January 1975.

Does Pentagastrin Suppress Secretin Induced Pepsin Secretion in Heidenhain Pouch Dogs?

In 1956 Schofield[1] showed that pepsin secretion from Heidenhain pouches fell following feeding. A possible explanation for this is that gastrin depresses pouch pepsin secretion. Vagne and Grossman[2] have since found that pepsin stimulated by distension of the intact stomach through a fistula is depressed by exogenous gastrin. It is not clear in their experiments how they avoided antral stimulation. Olbe et al.[3] have noted that pepsin stimulated from Pavlov pouches by sham-feeding is also depressed by suprathreshold doses of gastrin. We[4,5] have found the same thing in distended Heidenhain pouches. Both secretin and methacholine are good stimulants of Heidenhain pouch pepsin in dogs. It was of interest to us to determine if pentagastrin diminished the action of either of these agents.



The effect of pentagastrin (PG) on secretin- (Sec) (1 unit/min i.v.) stimulated pepsin and of secretin (Sec) on methacholine-(Meth) (2 µg/min i.v.) stimulated pepsin compared with secretin (Sec) alone and pentagastrin (PG) alone in Heidenhain pouches. *n* in PG experiments = 6; in others = 10.

In reviewing data obtained 3 or 4 years ago and presented here we have found that if progressively increasing doses of pentagastrin (Peptavalon®, kindly supplied by Ayerst Laboratories) (PG) (0.5, 1 and 2 µg/min i.v.) are added to a continuous i.v. infusion of secretin, 1 U/min, the stimulated pepsin secretion is progressively depressed (Figure). In the methacholine experiments a continuous background i.v. infusion of 2 µg/min methacholine was given and then 0.5, 1 and 2 units of secretin/min was superimposed. At the lowest dose only, the secretin response was significantly augmented by methacholine. Beyond this there was no significant difference. Certainly secretin did not depress methacholine-stimulated pepsin or vice versa. In fact, the calculated maximal responses and the slopes of the dose response curves (reciprocal plot) for secretin alone and with methacholine are not significantly different.

We have noted previously that pentagastrin does not stimulate Heidenhain pouch pepsin and that in many respects the actions of cholinergics and secretin on pouch pepsin resemble one another[5].

In the PG experiments, 6 dogs were used and in the methacholine experiments 10 were used. The results shown (Figure) are the means of the last two 10-min collections at each dose level. Pepsin was estimated using Anson's[6] hemoglobin method. The units are mg tyrosine liberated per 10 min collection.

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