K^+, 0.82 mM Mg^{++}) spread; the majority of them degenerated quickly, and the few surviving cells were poorly-differentiated myoblasts. Treatment with ionophore A 23187 facilitated attachment and spreading of cells cultured in Ca^{++}-free medium. About 30% of the treated cells spread (figure 3, table). A few neurites appeared after 5 days of culture. In myoblasts cross-striation was clearly seen on the 5th day, preceding formation of striated myofibrils in control cells cultured without Ca^{++}.

These stimulating effects of the ionophore on spreading and differentiation of cells cultured without Ca^{++} in the extracellular medium, could result from the release of intracellularly bound Ca^{++} from yolk platelets[13,19] and/or mitochondria[8] promoted by the ionophore A 23187. These experiments show that at the neurula stage there is a necessity for Ca^{++}, however there is already a different dependence of the various as yet morphologically undifferentiated cell-types, towards cations and principally Ca^{++}.

III. Effects of papaverine on this embryonic system. To complete these observations on the action of the ionophore, experiments were carried out with papaverine, which promotes Ca^{++} efflux.

The treatment by papaverine had no marked inhibitory effect on the attachment and spreading of cells. This suggests that papaverine does not alter the sites on the plasmalemma involved in cellular adhesion and spreading. Later on, papaverine treatment (only 1 h at the beginning of the culture) caused morphological modifications which were maintained during the whole life of the treated cells: yolk utilization was delayed (figures 6 and 7; table) and there were various nuclear and cytoplasmic alterations (figure 7). These modified cells did not degenerate and remained attached to the support when the culture-chamber was turned over, maintaining this new position for several weeks. Such cells survived for more than 1 month.

After about 3 weeks, there was a slight progress in differentiation but yolk utilization was still considerably slowed down. In neural cells, short and dumpy-looking axons appeared; myoblasts were fusiform and showed delayed and imprecise striation, masked by abundant yolk platelets; there were few melanophores (table). The cells degenerated after only 4 or 5 weeks.

Here again, papaverine disturbed, but did not change, the cell differentiation pathway. All the cell-types seen in the controls were also present in the treated cultures, indicating no differential inhibition of differentiation.

The effects of ionophore A 23187 and papaverine were quite irreversible since the duration of treatment was only 1 h (or 5 h) and the cells were then passed through 3 washes and maintained in standard Barth-medium for several weeks.

The experimental studies reported here provide evidence for differential cation (Ca^{++}) dependence of different types of cells in the amphibian neurula. Furthermore, they demonstrate that Ca^{++} does not alter cell determination at this stage.

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α-L-Aspartyl-L-alanine (a neural dipeptide) enhances synaptic transmission

R. Lim[1], S.T. Cheung and J.W. Crayton

Brain Research Institute, and Departments of Surgery (Neurosurgery) and Psychiatry, The University of Chicago, Chicago (Illinois 60637, USA), 18 June 1980

Summary. α-L-aspartyl-L-alanine, a dipeptide found in the brain, increases the amplitude of the miniature endplate potentials (MEPPs) in phrenic nerve-diaphragm preparations from rats. The peptide also stimulates the appearance of a population of 'giant' MEPPs.

The peptide, α-L-aspartyl-L-alanine, was recently identified by us[2] in the pig brain. As an initial step in determining whether this substance may have physiological activity in the nervous system, we studied its effect on spontaneous quantal release of acetylcholine at the neuromuscular junction in phrenic nerve-diaphragm preparations from rats. α-L-aspartyl-L-alanine increased the amplitude of the miniature endplate potentials (MEPPs) when used at 10^{-5} M concentration (figure 1). There was a shift in the mean amplitude of the regular MEPPs from 0.45 to

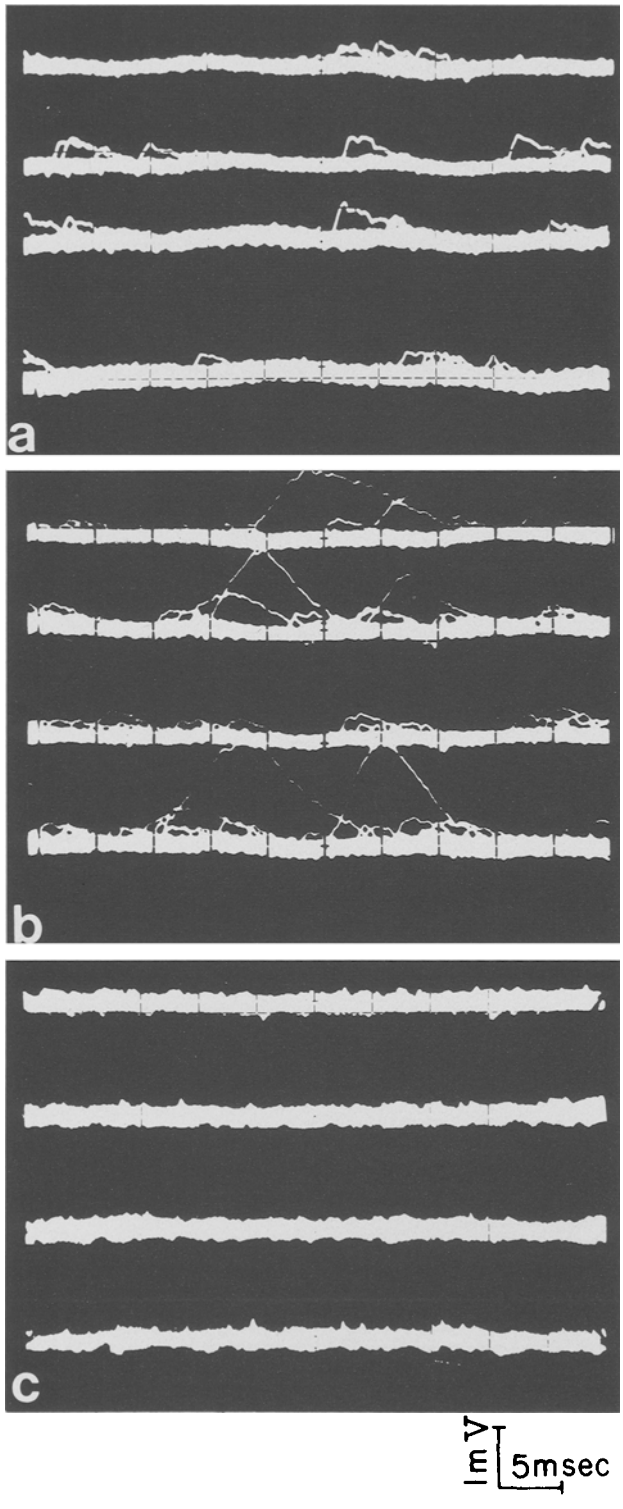

Fig. 1. Effects of α-L-aspartyl-L-alanine on MEPPs of rat diaphragm. *a* Before addition of peptide; *b* 15 min after addition of peptide; *c* 15 min after addition of d-tubocurarine. The peptide was obtained as a synthetic product from Vega Biochemicals, Tucson, Arizona. Diaphragms were removed from male Sprague-Dawley rats (150 g b.wt) and cut into 1 × 1 cm sections with nerve terminals attached to the center. Each section was incubated at 32 °C in a 15-ml bath through which was circulated a 200-ml Tyrode solution under constant oxygenation (5% CO_2 in O_2). Following a 30-min equilibration and a 60-min recording, the peptide was added to the bath at 10^{-5} M and the diaphragm was equilibrated for an additional 15 min before recording for the peptide effect. In some experiments, d-tubocurarine (1 mM) was added to the peptide-treated diaphragm and the disappearance of both classes of MEPPs was noted. Randomly selected and equally dispersed muscle fibres were impaled with glass micropipettes filled with 3 M KCl. Resistances were between 4 and 15 mΩ. Signals were amplified and displayed on an oscilloscope. Each tracing shown is a composite of 20 sweeps.

0.60 mV (figure 2). In addition, a population of 'giant' MEPPs with mean amplitude of 1.0 mV appeared (figure 2).

That the giant MEPPs were not brought about by local changes in muscle membrane potential was confirmed by their absence from the non-endplate regions of the muscle fibres. Furthermore, by adding d-tubocurarine in graded doses to the incubation bath, both the giant MEPPs and the 0.45–0.6 mV MEPPs were reduced in amplitude. At 1 mM, d-tubocurarine completely abolished all the endplate potentials (figure 1, C).

The increase in the amplitude of MEPP was sustained for at least 120 min in the continuous presence of the dipeptide, whereas the frequency showed no change during the same period. Interestingly, even when the peptide was washed out from the incubation bath after the first 30 min, the increase in amplitude persisted for at least 1 h. Increasing the peptide concentration from 10^{-5} M did not further increase the amplitude. At 10^{-6} M, the change was not detectable. The possibility that the effects of the peptide may be mediated by one or both of its degradation products, aspartic acid and alanine, was ruled out by the failure of these individual amino acids to affect the MEPPs at comparable concentrations. The stimulating effect of the peptide was not reproduced by L-alanyl-L-aspartic acid and β-L-aspartyl-L-alanine,indicating that the amino acid sequence as well as the type of peptide linkage is specific for its action. Another dipeptide, α-L-aspartyl-glycine, which was found in the urine[3] but has not been identified in the brain, also had no effect.

The shift in the mean amplitude of MEPP could be due to an increase in quantal size by packaging of more acetylcholine into each vesicle. Alternatively, it could be a postsynaptic event explainable by an increase in receptor sensitivity or a change in ion-conductance. The exact mechanism remains to be clarified. The possibility of inhibition of

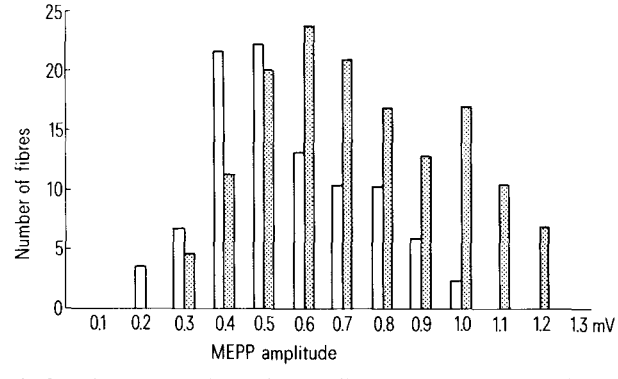

Fig. 2. Histograms of amplitude distribution of MEPPs before (empty bars) and after (filled bars) exposure to α-L-aspartyl-L-alanine. The experiment was conducted as described for figure 1. MEPP amplitudes were corrected for non-linearity to a reference potential of 85 mV. A total of 90 fibres from 9 diaphragms were recorded before addition of peptide, with a mean amplitude of 0.59 ± 0.04 mV (SD). A total of 140 fibres from 36 diaphragms were recorded over a 2-h period after addition of the peptide, with a mean amplitude of 0.78 ± 0.13 mV (SD), $p. < 0.001$.

acetylcholinesterase is ruled out by the absence of anticholinesterase activity in the peptide, as indicated in the table.
The observed giant MEPPs were about twice the amplitude of the regular MEPPs and suggest that the contents of 2 regular MEPPs were discharged at the same time, either by the fusion of 2 synaptic vesicles prior to the release[5] or by a 'drag' effect where the release of one vesicle leads to the immediate discharge of another[6].
These results suggest that α-L-aspartyl-L-alanine may have a physiological role in synaptic transmission. The high concentration needed and the long-lasting effect of the dipeptide may imply that it is a relatively weak modulator of cholinergic transmission with a slow but persistent action. It is interesting to note that the dipeptide is a part of the sequence of many naturally occurring peptides, including VIP (vasoactive intestinal peptide), ACTH 18–39, DSIP (delta-sleep-inducing-peptide) and eledoisin.
Basic knowledge of neurotransmission has traditionally accrued mainly from studies on peripheral systems. The usefulness of the phrenic nerve-diaphragm preparation as a prototype for central cholinergic transmission has been repeatedly confirmed. The current experiment demonstrates that this simple system can also be exploited for the study of modulators of the cholinergic system. Since the brain probably contains numerous peptides, many of which have yet to be identified, the inclusion of the nerve-muscle preparation as a first step in the investigation of putative neuromodulators may facilitate the delineation of the functional role of these substances.

Effect of α-L-aspartyl-L-alanine (asp-ala) on cholinesterases

Enzyme tested	Substrate hydrolyzed (nmole/min/incubation tube)
Acetylcholinesterase (4 µg/tube)	6.72 ± 0.34
Acetylcholinesterase (4 µg/tube) + asp-ala (10^{-5} M)	6.82 ± 0.27
Butyrylcholinesterase (2 µg/tube)	7.66 ± 0.26
Butyrylcholinesterase (2 µg/tube) + asp-ala (10^{-5} M)	7.78 ± 0.35

Erythrocyte acetylcholinesterase and serum butyrylcholinesterase (both from Sigma Chemical Co.) were assayed by the dithiobisnitrobenzoate method[4] using acetylthiocholine and butyrylthiocholine as the respective substrates. The enzyme concentrations were chosen so that the products at the endpoint of incubation fell within the linear portion of the dose-response curve. Where indicated asp-ala was added simultaneously with the enzyme. Results are mean of 5 assays ± SD. Statistical analysis shows no significant difference between results in the presence and absence of the peptide.

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Fourier analysis and spatial representation in the visual cortex

J.J. Kulikowski and P.O. Bishop[1]

Ophthalmic Optics Department, U.M.I.S.T., P.O. Box 88, Manchester M60 1QD (England), and Department of Physiology, John Curtin School of Medical Research, Australian National University, P.O. Box 334, Canberra City, 2601 (Australia),
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Summary. Simple cells in the cat visual cortex are shown to be general purpose analyzers of visual information achieving, at the same time, minimum uncertainty in spatial localization and spatial frequency. Their responses to moving bars, edges and gratings are linearly interrelated and predictable from each other.

Two different approaches to an understanding of the operation of the visual cortex have developed over the past 15 years. One approach, based on the pioneering work of Hubel and Wiesel[2], has concentrated attention on the spatial organization of cortical receptive fields and lines and edges have come to be regarded as the elementary features extracted by simple cells. The alternative approach is based on the application of spatial frequency (Fourier) methods and, by concentrating attention on the sensitivity of cortical neurons to sinusoidal gratings of varying spatial frequencies[3,4], this approach has tended to neglect discrimination of spatial position. It must be stressed however that, for simple cells operating within their linear range, both descriptions are mathematically equivalent: the bar (line) and edge detectors can be represented as cosine and sine components of the Fourier transform[5–7], as was shown earlier by Kulikowski and King-Smith[8] when evaluating line and edge detectors in psychophysical experiments. The present preliminary report aims not only to integrate the 2 approaches mentioned above but also to take into consideration, along lines suggested by Gabor's theory of communications[9,10], the ability of simple cortical cells to localize signals both in spatial position as well as in spatial frequency. Gabor's theory leads to the idea that the visual system attempts to analyze visual information most economically by using pairs of receptive fields of symmetrical (cosine) and antisymmetrical (sine) response profiles, to achieve minimum uncertainty in both spatial localization and spatial frequency. Whereas a symmetrical response profile (figure 1, Ba) has a minimum of 3 subregions (a centrally-located subregion, either ON or OFF, flanked by 2 weaker antagonistic areas), the antisymmetrical profile (figure 1, Ca) has a minimum of 4 subregions (2 approximately equal but antagonistic subregions, one ON the other OFF, located to either side of the centre, each flanked in turn by a very weak antagonistic area[8,11].
If simple cells are amenable to linear analysis[6], the inverse Fourier transform of the spatial frequency tuning curve should predict the spatial response profile. In previous reports[6,7] comparisons have been made between tuning curves prepared with moving gratings and response profiles obtained with stationary flashing stimuli. In our analysis, however, moving stimuli were used in both cases including response profiles to both moving lines (bars) and edges.