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Specificity of adenosine on transmitter output at the neuromuscular junction

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It has recently been found that the amount of transmitter released from the phrenic nerve of the rat, as measured by the ratio of the amplitude of evoked to spontaneous end-plate potentials, is reduced in the presence of adenosine, in a concentration of 0·025 mM or above (Ginsborg & Hirst, 1972). Investigations have now been made of the effects of a number of substances which might be expected to share some of the pharmacological properties of adenosine (see Burnstock, 1972). Of these only 5′-adenosine monophosphate (5′-AMP) shared the action of adenosine. The remainder, adenine, inosine, guanosine, cystine and uridine, in concentrations of up to at least 1 mM, did not reduce either the quantal content of end-plate potentials or alternatively the twitch tension of indirectly stimulated rat diaphragms bathed in high Mg^{2+}/low Ca^{2+} solutions: they were thus presumably without effect on transmitter release.

The interest in these results is related to the fact that adenosine and 5′-AMP are known to increase cyclic 3′,5′-adenosine monophosphate (cyclic AMP) in central nervous tissue whereas the remaining substances tested in these experiments are known not to have this effect (Sattin & Rall, 1970). The possibility that cyclic AMP is involved in the effect of adenosine on transmitter release or that the effect of adenosine on transmitter release and cyclic AMP have a common step cannot yet be rejected.

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Bicuculline and frog spinal neurones

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Bicuculline has been reported to antagonize selectively the inhibitory effect of γ-aminobutyric acid (GABA) on mammalian spinal neurones (Curtis, Duggan, Felix & Johnston, 1970). Both GABA and glycine depress ventral root responses to dorsal root stimulation in amphibian spinal cord (Curtis, Phillis & Watkins, 1961). In the present experiments some preliminary attempts have been made to see whether these effects show a differential sensitivity to bicuculline.

Experiments were performed on the isolated spinal cord of the frog (*Rana temporaria*) maintained in oxygenated frog Ringer solution at 15° C. A lumbar dorsal root (DR) was stimulated with single supramaximal shocks. Evoked potentials were recorded from the corresponding ventral root (VR) using platinum wire electrodes and DC amplification.

In the absence of added amino acid, bicuculline (5×10^{-5}M and upwards) produced activity in both ventral and dorsal roots, and augmented the VR response to DR stimulation. Induced activity was abolished by adding 5 to 10 mM Mg.

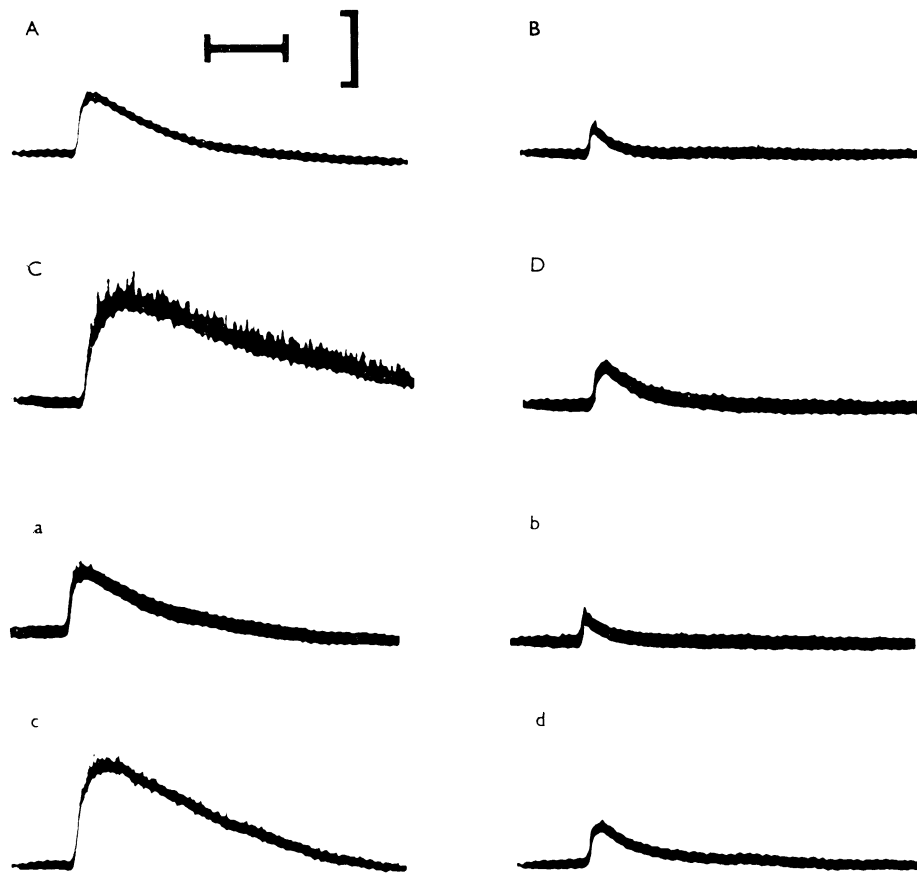

FIG. 1 Preparation pre-treated with magnesium chloride 3·5 mM. A-D, effect of bicuculline upon the depressant action of GABA on the electrically evoked ventral root response. A, Control. B, Maximum depression produced by 30 s exposure to $1{\cdot}02 \times 10^{-3}$M, GABA. C, 30 min after initial exposure to $1{\cdot}3 \times 10^{-4}$M bicuculline. D, Maximum depression produced in 45 s by the test dose of GABA during the action of bicuculline.

a-d, Effect of bicuculline upon the depressant action of glycine on the electrically evoked ventral root response in the same preparation as A-D. a, Control. b, Maximum depression produced by 30 s exposure to glycine $3{\cdot}5 \times 10^{-4}$ M.C. 45 min after initial exposure to $1{\cdot}3 \times 10^{-4}$M bicuculline. d, Maximum depression produced by 45 s exposure to the test dose of glycine during the action of bicuculline ($1{\cdot}3 \times 10^{-4}$M).

Calibration, A-D and a-d, 200 μV, 300 ms.

The action of amino acids on VR responses to DR stimulation were recorded in 3·5 mM Mg Ringer solution to reduce synaptically-mediated interneuronal effects. Under these conditions, direct effects on motoneurones predominate (Barany, 1948).

In the presence of added Mg, bicuculline still enhanced the evoked VR responses but, even at 10^{-4}M, failed to antagonize the depressant effects of GABA or of glycine (Fig. 1).

It is concluded that the increased excitability of amphibian cord following bicuculline may be dissociated from antagonism of the depressant effects of the inhibitory transmitters GABA and glycine.

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Action of γ-aminobutyric acid (GABA) on rat sympathetic ganglion cells

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Contrary to its usual hyperpolarizing action on central neurones, GABA depolarizes sympathetic ganglia (de Groat, 1970; Bowery & Brown, 1972). We have studied the mechanism of this action in isolated rat superior cervical ganglia using at room temperature conventional intracellular recording techniques.

All cells impaled responded to 100 μM GABA. This concentration produced a near maximal response. Higher concentrations produced rapid desensitization. Cells with recorded resting membrane potentials (E_m)>42 mV responded with depolarization, which could be reversed to hyperpolarization by passing steady depolarizing current. Cells with E_m<45 mV were usually hyperpolarized, which in turn could be reversed by hyperpolarizing currents. Cells hyperpolarized by GABA were depolarized by carbachol.

Both depolarization and hyperpolarization were accompanied (Fig. 1) by (i) failure of direct or orthodromic action potentials or a reduction of their positive overshoots, (ii) depression of the synaptic potential, and (iii) a large fall in the input resistance to hyperpolarizing current pulses.

The variation of potential change with E_m suggests a reversal potential for GABA action (E_{GABA}) of about −42 mV. A similar value was obtained from the intersection of extrapolated current-voltage curves for hyperpolarizing pulses measured at rest and

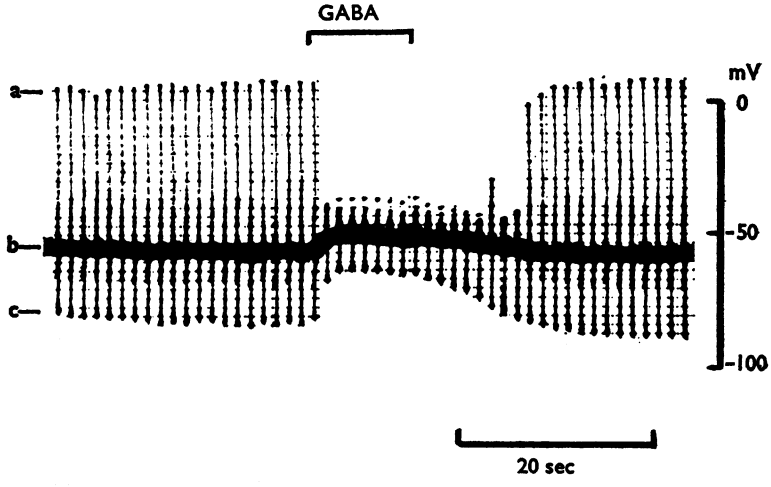

FIG. 1. Intracellular record from a neurone in an isolated rat superior cervical ganglion showing the effect of a 10 s application of GABA (100 μM) on (a) action potentials recorded in response to preganglionic nerve stimuli, (b) membrane potential, and (c) potential deflexions produced by applying hyperpolarizing current pulses (70 ms duration, 0·33 nA) 175 ms after each preganglionic nerve shock. During the exposure to GABA the orthodromic spike failed, leaving a synaptic potential; the membrane potential was reduced from −55 to −48 mV and the apparent input resistance reduced from 85 MΩ to 36 MΩ.