Amino acid receptors on frog spinal motoneurones

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The isolated hemisected amphibian spinal cord is a convenient preparation for demonstrating the action of neuro-active amino acids (Curtis, Phillis & Watkins, 1961). However, changes in motoneurone activity, as recorded from the ventral roots, reflect not only the direct action of applied substances but also the action of substances on neurones which synapse with motoneurones. Thus, the precise site of action of the applied substances is unknown. To overcome this difficulty, we have blocked regenerative activity with procaine, so allowing the recording of electrotonic propagation in the ventral root to be used as a direct measure of the action of the substances on the motoneurone membrane. With this system L-glutamate and L-aspartate depolarized motoneurones, while γ-aminobutyric acid (GABA) and taurine caused hyperpolarization. The action of GABA was blocked by picrotoxin and bicuculline, but not by strychnine. The action of taurine was blocked by strychnine but not by picrotoxin. Glycine caused a weak depolarization, and neither strychnine nor picrotoxin antagonized this action.

No specific blocking agents are yet available to enable a comparison to be made of the receptors for excitatory amino acids on frog motoneurones with those present on neurones in the mammalian central nervous system. However, a series of excitants covering a wide range of potencies might be expected to show the same order of activity on frog motoneurones as on mammalian spinal neurones if the receptors were similar. Eight such substances (including three new excitants, marked with an asterisk, which have not been previously studied) were tested and the order of excitatory potency was found to be: kainate > N-methyl-D-aspartate ≃ DL-2-amino-4-thiosulphonylbutyrate (thiohomocysteic acid)* ≃ DL-homocysteate > L-glutamate ≃ L-aspartate ≃ 6-hydroxy-2-pyridylalanine (6-H-2PA)* > L-2-amino-3-thiosulphonylpropionate (thiocysteic acid)*. The same order of potency was found for rat spinal interneurones when the substances were administered by microelectrophoresis (Biscoe, Headley, Martin & Watkins, unpublished observations). Moreover, in the frog cord experiments, all the compounds gave a log dose-response plot parallel to that for L-glutamate.

The close similarity between the excitatory amino acid receptors on frog and rat spinal neurones which is apparent from these studies contrasts with the results of structure-activity studies for glutamate agonists on invertebrate preparations, where, for example, kainate and DL-homocysteate have been found to have only weak excitatory actions (Clements & May, 1974). It seems likely that results of further investigations using this amphibian system, including a search for glutamate antagonists, will be relevant to the mammalian central nervous system.

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Sodium and the response of rat descending colon and rat uterus to angiotensin II

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Angiotensin produces a contraction of most isolated smooth muscle preparations (Gross, 1971) and in lower concentrations stimulates transepithelial sodium transport (Crocker, 1971). It has been reported that the contractile action of angiotensin on guinea-pig ileum is dependent upon the extracellular sodium concentration (Blair-West, Harding & McKenzie, 1967). In the present study the role of Na^+ has been investigated on the contractile response of rat colon and uterus to angiotensin by altering the Na^+ concentration of the Tyrode solution and by the use of inhibitors of Na^+ movement.

Muscle preparations, from Wistar rats, were suspended in Tyrode solution and gassed with air. Contractions were measured at the maximum sustained deviation from resting tension using

concentrations of acetylcholine and angiotensin which produced responses approximately the same size as 50% maximum acetylcholine response.

When the Na^+ concentration of the Tyrode solution was varied from the normal concentration of 137 mmol the responses of rat colon to acetylcholine and to angiotensin changed similarly being reduced by 12% at 120 mmol and 50% at 68.5 mmol Na^+ and reduced by 30% at 171 mmol and 60% at 205.5 mmol Na^+. At high concentrations of ouabain (1.0 mmol) there was equal reduction of responses to acetylcholine and to angiotensin of both rat colon (37%) and rat uterus (57%). After 0.1 mmol ethacrynic acid, the isometric responses of rat colon to acetylcholine were reduced by 15.4 ± 0.7% while the angiotensin response was reduced by 42.9 ± 5.3% ($n = 6$, p 0.001). With rat uterus, it was necessary to record responses isotonically (1 g load), there was equal reduction (39%) of responses to acetylcholine and to angiotensin. However, with 3 g load, there was a significantly greater reduction ($n = 7$, P 0.01) of the angiotensin response (60.0 ± 3.6%) compared with the acetylcholine response (35.8 ± 4.4%).

Therefore, a preferential reduction of the angiotensin response of uterus was observed only under conditions of high energy expenditure. Since it has been reported that ethacrynic acid inhibits cellular energy production (Epstein, 1972) it appears that its effect on uterus might be due to this mechanism rather than an effect on Na^+ movement. This conclusion is supported by the severe inhibition of all contractile responses observed during exposure of rat uterus to higher concentrations of ethacrynic acid (0.5 mmol). It is also consistent with our previous observations that the angiotensin response of these tissues involves an ATP-dependent step (Crocker & Wilson, 1975; Wilson, Crocker & Willavoys, 1974). The present findings, however, do not support a specific role for Na^+ in the interaction of angiotensin with these tissues.

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A study of tetramethylenedisulphotetramine (TETS) and related compounds as antagonists of presynaptic inhibition and microiontophoretically applied γ-aminobutyric acid (GABA) and glycine in the rat cuneate nucleus

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TETS is an extremely potent convulsant that antagonizes the actions of GABA at the rat superior cervical ganglion (Bowery, Brown & Collins, 1975) and at the crustacean neuromuscular junction (Large, 1975). However, these preparations are insensitive to glycine and so give no indication of the specificity of TETS as a GABA antagonist. We have therefore examined the specificity of TETS and its effect on presynaptic inhibition in the rat cuneate nucleus. In addition, three other structurally related compounds, (Figure 1), have been examined.

Experiments were performed on 14 rats anaesthetized with urethane and prepared as described by Hill & Miller (1974). Presynaptic inhibition was estimated from the amplitude of the P-wave component of the cuneate field potential produced by stimulation of the appropriate forepaw (Anderson, Eccles, Schmidt &