Br. J. Pharmac. (1977), **61**, 217–222

SPASMOGENIC AND POTENTIATING ACTIONS OF SOME AMINO ACIDS ON THE GUINEA-PIG MYOMETRIUM

J.R. BEDWANI, M. ISHIZAWA[1], V.R. PICKLES & SURANG SUWANKRUGHASN[2]
Department of Physiology, University College, Cardiff CF1 1XL

1 Thirty-three amino acids were applied separately in concentrations of 2 to 10 mM to guinea-pig uterine horns *in vitro* at pH 7.4. About half the acids regularly produced contractions.

2 Glycine and the straight-chain L-α-amino acids up to norleucine were active (longer ones not tested); D-isomers were less potent or inactive in these concentrations. The ω-amino acids γ-aminobutyric acid (GABA) and δ-aminovaleric, and the α,ω-diamino acids L-α, β-diaminopropionic and L-α,γ-diaminobutyric were active, whereas others of similar chain-length such as β-alanine and lysine were not. The diacidic acids, glutamic and homocysteic, were more active than the amido-amino acids, glutamine and asparagine. Histidine and phenylalanine showed little or no activity.

3 The use of appropriate blocking agents indicated that the responses to representative acids were not mediated by histamine, 5-hydroxytryptamine, acetylcholine, noradrenaline or by prostaglandins. Attempts to block the actions of glycine and GABA with strychnine, thebaine, picrotoxin, bicuculline or tetramethylenedisulphotetramine (TETS) were unsuccessful.

4 When some of the acids that were spasmogenic at 2 to 10 mM were applied at sub-spasmogenic doses, they transiently potentiated other spasmogens such as oxytocin or acetylcholine. This effect was also shown by a mixture of amino acids at approximately the normal plasma concentrations.

5 There is some similarity between the spasmogenic activities of different amino acids and their known abilities to depolarize neurones.

Introduction

Amino acids may stimulate smooth muscle (Jones, 1962; Klingenberg, 1966; Lewis, McMartin, Rosenthal & Yates, 1972). Among these, the only extensive study of amino acids in general was that of Lewis *et al.* (1972), who were concerned to identify the non-histamine active substances in the stratum corneum of human skin, and to define the receptors on which the substances acted; these authors used the guinea-pig ileum as their test object. We, however, wished to study a possible parallelism between the actions of amino acids in stimulating smooth muscle, and in depolarizing cells in the central nervous system (CNS). Consequently, we have compared several acids known to be active on CNS cells with others not known to be active. Preliminary trials suggested that the guinea-pig uterus was a suitable test object. Some of the results have been demonstrated to the British Pharmacological Society (Ishizawa & Pickles, 1975) or communicated to the Physiological Society (Bedwani, Pickles & Suwankrughasn, 1976).

[1] Present address: Department of Physiology, Sapporo Medical School, S.1, W.17, Sapporo, Japan.
[2] Present address: Department of Physiology, Chiengmai University, Chiengmai, Thailand.

Methods

Uterine horns from non-oestrous guinea-pigs weighing 420–650 g were suspended in a modified Krebs-Henseleit solution at pH 7.4, maintained at 36–37°C and normally containing Ca^{2+} and Mg^{2+} each 1 mM, the $[Mg^{2+}]$ being increased if necessary to give a conveniently low degree of spontaneous activity in the preparations. Contractions were recorded either isometrically or by means of a lightly spring-loaded lever on a kymograph. The amino acids or their sodium salts or hydrochlorides (Sigma Chemical Co.) were freshly made up as isotonic solutions in deionized water and adjusted if necessary to pH 7.4. Volumes of these solutions from 1/50 to 1/10 of the organ-bath volume were added directly to give the higher concentrations of amino acids; for the lower concentrations, the original amino acid solutions were diluted ten-fold with isotonic NaCl solution. Control

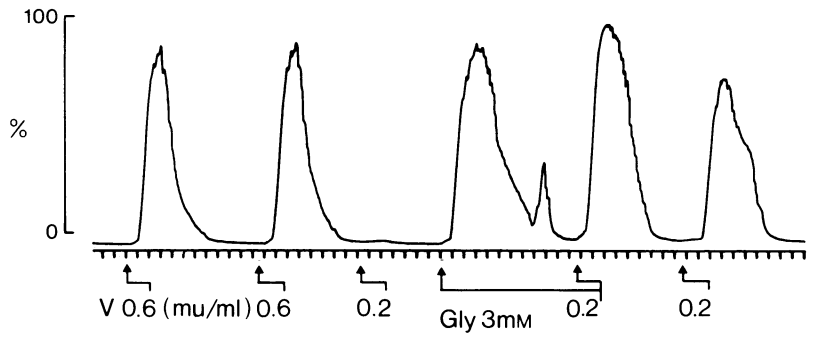

Figure 1 Guinea-pig uterine horn *in vitro*: effects of glycine (3 mM bath concentration) and vasopressin (V, doses as indicated) alone and in the presence of glycine. Time-marker 1 minute. On vertical scale, 100% =47 mm.

applications of NaCl or isotonic sucrose solution had no effect. Tests with vasopressin or oxytocin were always included as a check of the excitability of the preparations. Each acid was tested in at least three separate experiments but some, especially glycine and γ-aminobutyric acid (GABA), in many more.

Results

Potency groups

A typical response to glycine, one of the more active of the amino acids tested, is shown in Figure 1. The responses of the guinea-pig myometrium in general are less precisely dose-dependent than those of the ileum, and smooth 'dose-response' curves may require a large number of trials. For our present purposes, we have simply grouped the acids broadly in terms of their relative potencies. Thus glycine fell into the most potent group, in which concentrations within the range 2 to 5 mM usually caused strong but not necessarily maximal contractions. In the less potent group, 5 to 10 mM was needed for a similar response; while for the least potent acids, 10 to 15 mM gave little or no response.

The potencies thus defined indicate the responses in most but not in all experiments. In a few experiments, an acid might give responses either greater or less than would be suggested by the potency-group to which it was assigned. Where this variability was particularly noticeable, it is indicated in Table 1 by a range of potency groups. Another cause of variability was the differences between uteri in their spontaneous contractile activity: preparations showing greater activity were more sensitive to amino acids but gave

Table 1 Concentrations of acids needed to produce contractions of the guinea-pig myometrium, as described in the text

α-Amino acids	*ω-Amino acids*	*α,ω-Diamino acids*
Glycine + +		
L-α-Alanine + +	β-Alanine 0	L-α,β-Diaminopropionic +
L-α-Aminobutyric + +	GABA 0 – + +	L-α,γ-Diaminobutyric + +
L-α-Aminovaleric (norvaline) +	δ-Aminovaleric + +	L-α, δ-Diaminovaleric (ornithine) 0
L-α-Aminocaproic (norleucine) + +	ϵ-Aminocaproic 0	L-α,ϵ-Diaminocaproic (lysine) 0
Branched-chain amino acids	*Diacidic amino acids*	*Amido amino acids*
α-Amino*iso*butyric +	L-Glutamic + –+ +	L-Glutamine 0
L-Valine 0	L-Aspartic 0	L-Asparagine 0
L-Leucine 0 – +	DL-Homocysteic +	
*Others, including non-amino acids**	*N*-acetyl-L-aspartic 0 – +	
β-Aminobutyric 0	Proline +	Serine +
β-Hydroxybutyric* 0	Histidine 0 – +	Kainic + +
γ-Hydroxybutyric* 0 – +	Phenylalanine 0	δ-Aminolaevulinic + +

Key: + + 2 to 5 mM; + 5 to 10 mM; 0 > 10 mM, or no response.

recordings that were more difficult to assess quantitatively. This was particularly well illustrated by a few experiments in which the two horns of the same uterus, treated identically so far as was known, nevertheless showed different degrees of spontaneous activity.

Potency in relation to structure

Table 1 shows the acids we tested, classified according to their chemical nature and with their potencies indicated in terms of these three groups.

All the neutral straight-chain L-α-amino acids we tested were active. Of the corresponding ω-amino acids from β-alanine to ε-aminocaproic acid, only δ-aminovaleric was regularly active, though GABA was equally potent in some experiments. The first two L-α,ω-diaminoacids, namely L-α,β-diaminopropionic and L-α,γ-diaminobutyric were active as might be expected; but surprisingly neither L-α,δ-diaminovaleric (ornithine) nor L-α,ε-diaminocaproic (lysine) was active in our normal experimental conditions. Three D-α-amino acids were tested, namely D-α-alanine, D-α-aminobutyric acid and D-norleucine, and all proved to be less active than their L-enantiomers; D-lysine was also inactive. The branched-chain acids α-amino*iso*butyric, L-valine and L-leucine were either inactive or less potent than the corresponding straight-chain acids.

Among diacidic substances, L-glutamic and DL-homocysteic were active; the activity of glutamic acid contrasted with the inactivity of glutamine. Aspartic acid, its amide asparagine, and *N*-acetyl-L-aspartic acid, were less potent than glutamic acid.

Table 1 also shows the results with a number of other acids, including proline (an imino acid) and others that are not amino acids. In comparisons on the same uteri, GABA was shown to be more potent than γ-hydroxybutyric, β-aminobutyric and β-hydroxybutyric acids. Kainic acid which is a powerful convulsant (Shinozaki & Konishi, 1970) seemed to be more potent than glutamic acid to which it is structurally related, but the amount available did not allow full testing.

Action not mediated by other spasmogens

In order to eliminate the possibility that the responses were mediated by the liberation of other spasmogens, representative amino acids were tested in the presence of the specific antagonists mepyramine (2 μM), hexamethonium (10 μM), atropine (1 μM), phentolamine (1.6 μM), and methysergide (0.4 μM). The myometria were then unresponsive to histamine, acetylcholine, noradrenaline and 5-hydroxytryptamine, but they still responded normally to glycine, α,γ-diaminobutyric acid, and δ-aminolaevulinic acid. Other experiments in which the antagonists were applied individually showed that responses to GABA were unaffected. Histamine responses were blocked by mepyramine but not by burimamide (20 μM), showing that they were mediated by H_1-receptors only, and in these circumstances it was not surprising that burimamide did not alter the responses to glycine. In two experiments, indomethacin (6 and 14 μM) was present throughout in the Krebs-Henseleit solution for one uterine horn while the other was used as a control; there was no difference in the direct responses to glycine, GABA or glutamate, suggesting that these responses were not mediated by endogenous prostaglandin production. The prostaglandin antagonist 7-oxa-prost-13-ynoic acid was spasmogenic on the myometrium, and another antagonist diphloretin phosphate (DPP) proved to be not highly specific on this tissue; consequently, clear evidence could not be obtained for or against the possible participation of previously formed prostaglandins in the amino acid responses.

Because some effects of glycine or GABA in the CNS may be blocked by strychnine, thebaine, picrotoxin, bicuculline or tetramethylenedisulphotetramine (TETS), these substances were used in attempts to block the action of glycine and GABA on the myometrium. The results were difficult to interpret, because 1 mM or even lower concentrations of these substances either caused non-specific depression or were themselves spasmogenic. However, no trace of specific antagonism was ever seen.

Potentiation of other spasmogens

Some amino acids were found to potentiate other spasmogens. An example is seen in Figure 1, where a previously sub-threshold dose of vasopressin, 0.2 mu/ml, gave a large response when added in the presence of glycine, and a smaller but definite one again 9 min after the glycine had been washed out of the organ bath. In neither instance can the effect be readily explained by simple summation of responses. Further examples are shown in Figure 2, where the direct responses to GABA and to glycine were small but the responses to vasopressin were markedly potentiated. Figure 3 shows the results of another series of experiments, in which GABA concentrations less than one-tenth of the threshold for direct responses potentiated the spasmogenic effect of added Ca^{2+}. Similar results have been obtained with glycine.

The phenomenon seen in Figure 1, in which the potentiation apparently out-lasted the presence of the amino acid in the organ bath, varied greatly from experiment to experiment and possibly from one amino acid to another. In a few instances, it was as marked and as prolonged as the 'enhancement' following the application of prostaglandin E_1 or E_2 to the guinea-pig myometrium (Clegg, Hall & Pickles, 1966); whereas in other experiments it was absent or led rapidly into a period of decreased responses. This variability was not studied in detail.

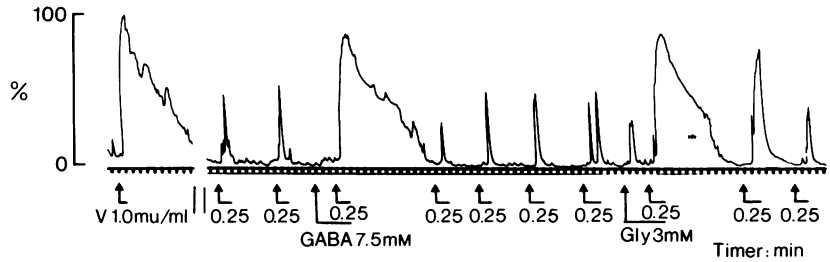

Figure 2 Guinea-pig uterine horn *in vitro*: responses to vasopressin (V, doses as indicated), and the effects of γ-aminobutyric acid (GABA) 7.5 mM and glycine (Gly) 3 mM on those responses. Gap between sections, 20 minutes. On vertical scale, 100% = 33 mm.

For a final short series of experiments two amino acid concentrates were prepared, one 'active' and the other 'inactive', such that on appropriate dilution the amino acid concentrations would approximate to those found free in the plasma of various species (Altman & Dittmer, 1974). The 'active' mixture comprised some of the acids given the + or + + rating in Table 1; on dilution it contained alanine 0.3, glycine 0.2, proline 0.2, serine 0.1, glutamic acid 0.05, norleucine 0.05 and α-aminobutyric acid 0.02 mM. Other acids rated + or + + were not included because their concentrations in plasma are much lower, or zero. The 'inactive' mixture on dilution gave lysine 0.15, leucine 0.1, histidine 0.05, ornithine 0.05, phenylalanine 0.05, and valine 0.02 mM. The results shown in Figure 4 were typical: the addition of the mixture of 'active' amino acids in plasma concentrations simultaneously with the oxytocin or acetylcholine increased the responses to the latter, but a similar addition of 'inactive' acids had no apparent effect. The 'inactive' mixture did not diminish the effect of the 'active' mixture.

More than half the amino acid content of the 'active' mixture consisted of glycine and alanine, both of which are among the most potent spasmogens. It might therefore be asked whether the potentiating effect of the 'active' mixture was largely or entirely due to either or both of these acids alone. However, an 'active' mixture from which the glycine and alanine had been omitted still gave definite potentiation, though less than that given by the complete mixture.

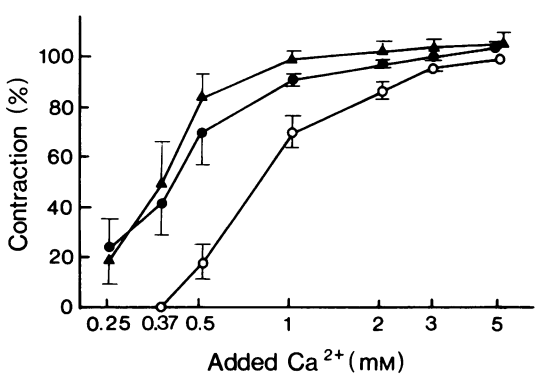

Figure 3 Guinea-pig uterine horns *in vitro* in a medium containing Ca^{2+} 0.1 mM and Mg^{2+} 3 mM, contracting in response to added $CaCl_2$ in the concentrations shown on the abscissa scale. (○) In absence of γ-aminobutyric acid (GABA), $n = 11$; (●) in presence of GABA 0.05 mM, $n = 5$; (▲) in presence of GABA 0.15 mM, $n = 5$. The contractions are expressed as percentages of the responses to the addition of 5 mM $CaCl_2$ in the absence of GABA. Vertical bars indicate ± s.e. mean.

Effects on spontaneous myometrial activity

When a myometrial preparation is contracting and relaxing spontaneously, it may be difficult or impossible to distinguish between a superimposed spasmogenesis as such, and an induced increase in the amount of spontaneous activity. In either case, the effect is difficult to quantitate. We have not systematically studied such effects in the experiments described here, but (as mentioned before) incidental observations have suggested that amino acids may be apparently more potent when assessed on a spontaneously active preparation than on a quiescent one. Figure 5 shows this particularly clearly, since the 'active' mixture alone normally had no apparent effect on quiescent preparations; and it also accords with the conclusions in the paragraph above.

Discussion

Our experiments on the guinea-pig uterus broadly confirm those of Lewis *et al.* (1972) on the ileum, in that a wide range of amino acids, but not all, can strongly stimulate smooth muscle when added in concentrations from 2 to 10 mM. Although it is difficult to make exact comparisons between the two investigations, there may be some significant differences

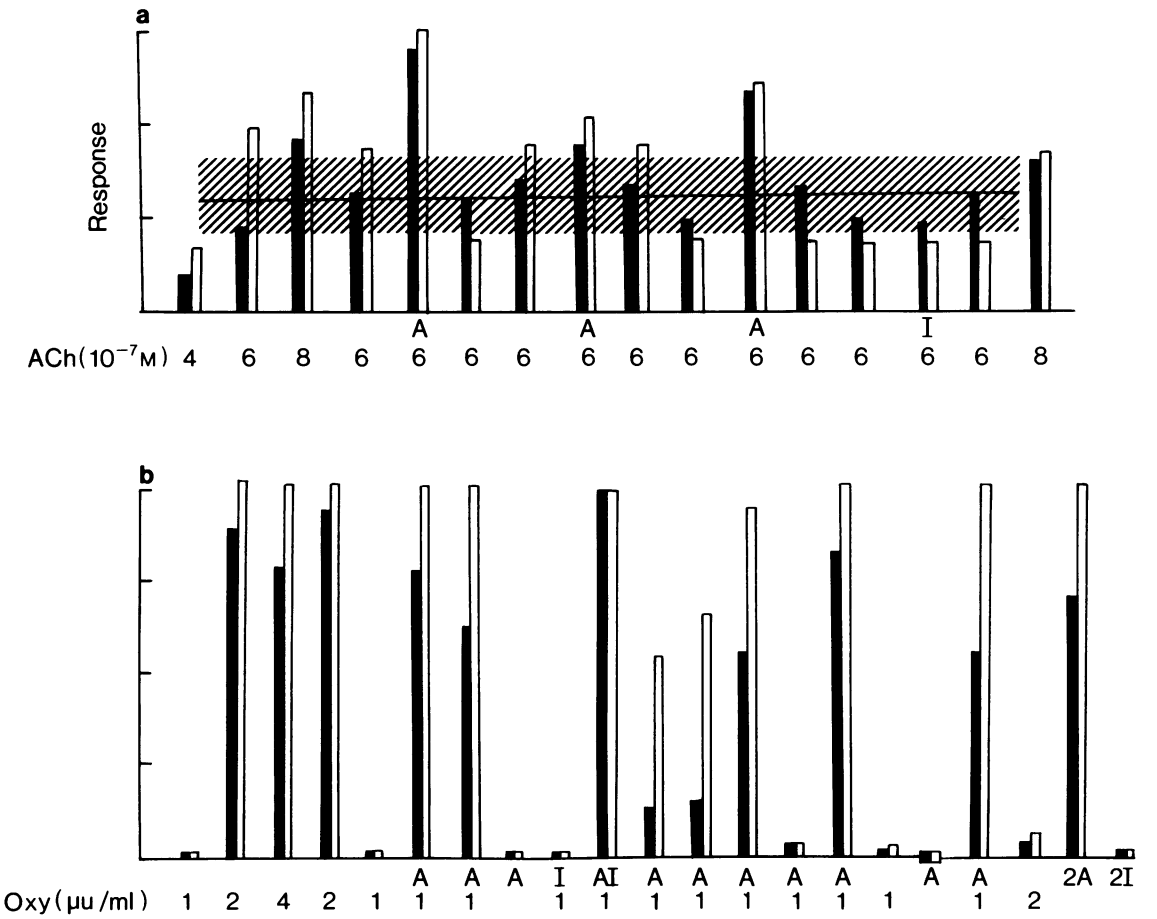

Figure 4 (a) Responses to acetylcholine (ACh) in the doses stated, with the simultaneous addition of the 'active' amino acid mixture (see text) at A, or the 'inactive' mixture at I. Each response is plotted both as the height on the recording (open column) and as the area under the curve from the time of application to 4 min after wash-out, when relaxation was nearly complete (solid column). The line, which is not quite horizontal, shows the calculated regression, on time, of the responses to the 6×10^{-7} M doses of acetylcholine alone (solid columns); the hatched area indicates ± s.d. (b) Responses of another preparation to oxytocin, expressed as in the upper graph. AI = simultaneous application of 'active' and 'inactive' mixtures; 2A, 2I = application of 'active' or 'inactive' mixture in double the previous concentration. The change from a very small to a near-maximal response with only a doubling of the dose, and the occasional apparently anomalous response such as the 14th one in (b), are characteristic of many guinea-pig uterine preparations *in vitro*. An apparent inhibitory phase, such as that following the application of A+I+oxytocin 1 (10th response), was seen in some other experiments but was not systematically investigated.

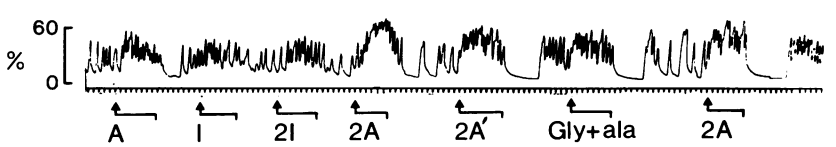

Figure 5 Effects on a spontaneously active preparation. A, I: 'active' and 'inactive' amino acid mixtures as before; A': as 'active' mixture but without glycine and alanine; Gly+ala: glycine 0.4 mM, alanine 0.6 mM, which when added to 2A' would make it the same as 2A. On vertical scale 60% = 12.6 mm.

in the results. In our experiments alanine was about twice as potent as glycine, whereas in theirs it was about seven times as potent; in our experiments glutamic acid was more potent than glutamine, whereas in theirs the reverse was true. The branched-chain acids, valine and leucine, which were of medium potency in their experiments were relatively inactive in ours. We tested homocysteic and kainic acids (which Lewis *et al.* (1972) did not test) because they are known to be depolarizers of central-nervous cells, and we found them active.

The use of specific blocking agents has shown that representative amino acids do not act by release of acetylcholine, noradrenaline, histamine or 5-hydroxytryptamine. Prostaglandin release is not a major factor though we cannot entirely exclude a minor effect of this kind. Lewis *et al.* (1972) postulated a receptor 'particularly sensitive to L-α-amino acids with a short side chain', GABA acting by a different mechanism. Our results are not incompatible with there being similar receptors in the myometrium; nevertheless, we are not positively proposing this. The range of active amino acids is wider and the effective concentrations much higher than one usually associates with specific action at receptors; for example, at post-synaptic glutamate receptors in the CNS. Other possible mechanisms may have to be considered. One might be of the type postulated by Klingenberg (1966), who suggested that cyclical fluctuations in a mechanism involving glutamate oxidation, Ca^{2+} storage and glutamine synthesis might explain the myometrial responses to exogenous glutamate. Alternatively the contractions might follow depolarization accompanying active uptake of amino acids, but we do not know of any studies of uptake in the myometrium with which to compare our results.

Such considerations are relevant to the comparison with the effects of amino acids on central neurones. For example, glutamate increases the activity of both types of cell, but with a great difference in effective dose. Glycine is best known as causing strychnine-sensitive hyperpolarization and inhibition in spinal cord neurones; but it may also cause strychnine-resistant depolarization probably leading to excitation (Nicoll, Padjen & Barker, 1976; Evans, Francis & Watkins, 1976), which corresponds more closely with its myometrial effect. The myometrium-stimulating potencies of various amino acids show a better correlation with their rates of metabolism by brain slices than with their uptake (Sadasivudu & Lajtha, 1970). We suggest that if the effects of amino acids on the myometrium correspond with any effect in the CNS, this is not with transmitter actions but with less specific effects which nevertheless are not given equally by all amino acids.

The concentrations of amino acids needed to produce definite contractions of the myometrium in the absence of other spasmogens are of the same order as those found free in whole-tissue extracts but are ten or more times as great as those in plasma. However, a mixture of acids in approximately the same concentrations as in plasma regularly potentiated other spasmogens. We propose that it is the excitability-modulating effect of some amino acids, rather than their direct spasmogenic action, that may represent a possible physiological effect on the myometrium.

We wish to thank Mr B. Shears and Mr P. Blanning for their skilled technical assistance. Gifts of 7-oxa-prost-13-ynoic acid from Dr J. Fried, DPP from Dr H. Fex, TETS from Dr J. F. Collins and kainic acid from Dr J. C. Watkins are also gratefully acknowledged.

References

ALTMAN, P.L. & DITTMER, D.S. (1974) (ed). *Biological Data Book*, 2nd Edition, pp. 1806–1811. Bethesda: Federation of American Societies for Experimental Biology.

BEDWANI, J.R., PICKLES, V.R. & SUWANKRUGHASN, S. (1976). Some amino acids as myometrial spasmogens. *J. Physiol., Lond.*, **260**, 62–63P.

CLEGG, P.C., HALL, W.J. & PICKLES, V.R. (1966). The action of ketonic prostaglandins on the guinea-pig myometrium. *J. Physiol., Lond.*, **183**, 123–144.

EVANS, R.H., FRANCIS, A.A. & WATKINS, J.C. (1976). Bimodal action of glycine on frog spinal motoneurones. *Brain Res.*, **118**, 395–401.

ISHIZAWA, M. & PICKLES, V.R. (1975). A comparison of some smooth-muscle effects of GABA and of prostaglandin E_1. *Br. J. Pharmac.*, **54**, 279–280P.

JONES, H.C. (1962). The action of L-glutamic acid and of structurally related compounds on the hind gut of the crayfish. *J. Physiol., Lond.*, **164**, 295–300.

KLINGENBERG, H.G. (1966). Erregung des glatten Muskels durch Fluorid und Glutaminsäure. *Z. Biol.*, **115**, 215–228.

LEWIS, G.P., McMARTIN, C., ROSENTHAL, S.R. & YATES, C. (1972). Isolation and identification of pharmacologically active amino acids in skin and their structure-activity relationship on the guinea-pig ileum. *Br. J. Pharmac.*, **45**, 104–117.

NICOLL, R.A., PADJEN, A. & BARKER, J.L. (1976). Analysis of amino acid responses of frog motoneurones. *Neuropharmac.*, **15**, 45–53.

SADASIVUDU, B. & LAJTHA, A. (1970). Metabolism of amino acids in incubated slices of mouse brain. *J. Neurochem.*, **17**, 1299–1311.

SHINOZAKI, H. & KONISHI, S. (1970). Actions of several anthelminthics and insecticides on rat cortical neurones. *Brain Res.*, **24**, 368–371.

(Received January 21, 1977. Revised April 1, 1977)