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THE ACTION OF L-GLUTAMIC ACID AND OF STRUCTURALLY RELATED COMPOUNDS ON THE HIND GUT OF THE CRAYFISH

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In the course of an investigation of the pharmacological properties of dog and guinea-pig brain extracts a factor was discovered which caused a stimulation of the isolated hind gut of the crayfish. Chromatography of purified extracts indicated that the excitatory factor consisted of glutamic and aspartic acids. These dicarboxylic amino acids have been extracted from mammalian brains by many previous workers (Cravioto, Massieu & Izquerdo, 1951; Ansell & Richter, 1954; Tallan, Moore & Stein, 1954; Porcellati & Thompson, 1957; van Harreveld, 1958). The pharmacological properties of glutamic acid and of related compounds have been studied on the mammalian spinal cord (Curtis, Phillis & Watkins, 1960; Curtis & Watkins, 1960), on the mammalian cerebral cortex (van Harreveld, 1958; Purpura, Girado, Smith, Callan & Grundfest, 1958), on the crayfish neuromuscular junction (van Harreveld, 1958; Robbins, 1958, 1959), and on the crayfish stretch receptor preparation (Elliot & Florey, 1956).

The isolated crayfish hind gut preparation was originally described by Florey (1954*a*, *b*) and in a recent paper (Florey, 1961) the action on this tissue of a range of compounds which included glutamic and aspartic acids was reported. Florey used the species *Cambarus clarkii* (syn. *Procambarus clarkii* Girard), *Orconectes virilis* Haegen and *Pacifastacus leniusculus*. However, in the present experiments on another species of crayfish somewhat different results were obtained, and this paper describes the effects of glutamic acid, aspartic acid and some structurally related compounds on the hind gut of *Astacus astacus* L. (syn. *Astacus fluviatilis* Fab.).

METHODS

The European species *Astacus astacus*, which was caught from the river Dorn, near Wootton, Oxfordshire, or purchased from a biological supply firm, was used in all the experiments. The body length was 5 cm or more, but in some experiments smaller specimens were used with only moderate success. The dorsal half of the exoskeleton was cut away, revealing the hind gut lying dorsal to the abdominal muscles. The gut was dissected free from the adjacent tissues except at the anal end, where a piece of the telson was left attached to it. A thread was tied to the telson fragment and another round the thoracic end of the intestine.

The preparation was then suspended in an organ-bath of volume 2·5 ml. A lightly loaded gimbal lever was used to record contractions on the kymograph.

The bathing medium used was that of van Harreveld (1936) and consisted of (g): NaCl 12·0; KCl 0·4; $CaCl_2$ 1·5; $MgCl_2$ 0·25; $NaHCO_3$ 0·2; and H_2O 1 l. The solution was aerated with a mixture of O_2 (95 %) and CO_2 (5 %), although the tissue was found to respond to drugs equally well when O_2 (100 %) was used. All experiments were carried out at room temperature.

Drugs were dissolved in crayfish saline solution or in distilled water, and were always diluted with crayfish saline solution. Doses were applied to the bath every 2 min and were left in contact with the tissue for 10–20 sec.

RESULTS

Response to drugs

Acetylcholine caused the preparation to contract at a threshold concentration of 10^{-8} g/ml. The response to acetylcholine was blocked by the acetylcholine antagonists hyoscine or lachesine (5×10^{-7} g/ml.), but not by D-tubocurarine up to 10^{-5} g/ml.

Histamine and adenosine phosphates had no effect on the tissue in concentrations of up to 10^{-4} g/ml. Substance P (3 units/ml.) was also ineffective.

Response to L-glutamic acid

L-Glutamic acid caused a contraction at a threshold concentration of 5×10^{-6} g/ml. The contraction was not blocked by acetylcholine antagonists. Figure 1 shows contractions due to doses of acetylcholine, L-glutamic acid and L-aspartic acid in a 2·5 ml. bath volume.

The contractions to L-glutamic acid were more transient than those to acetylcholine, lasting only 5 sec, and relaxation occurred before the acid was washed out of the organ-bath. Acetylcholine on the other hand caused a contraction which was maintained until the bath fluid was replaced.

The action of compounds structurally related to L-glutamic acid

A range of compounds structurally akin to L-glutamic acid was tested on the preparation. The results are summarized in Table 1.

Configuration. D-Glutamic acid was found to be *ca.* 10 times less active than the L-derivative.

Length of the carbon chain. The activity of four dicarboxylic α-amino acids was investigated. Of these L-glutamic acid (C_5) was the most active. L-Aspartic acid (C_4) was 2–3 times less active; the racemic DL-α-amino adipic acid (C_6) was 10 times less active. DL-α-Aminopimelic acid (C_7) had no action.

Removal of the acidic groups. It was found that neither the monoamide nor the diamide derivatives of L-glutamic acid and L-aspartic acid had any stimulating activity when tested at concentrations up to 10^{-4} g/ml.

Relative disposition of amino and carboxyl groups and introduction of second amino groups. It was found that removal of the amino group from the α to the β position (β-aminoglutaric acid) caused complete loss of the stimulating activity of glutamic acid. Also, it was shown that the introduction of a second amino group into DL-glutamic acid (DL-α-diaminoglutaric acid) or aspartic acid (α-β-diaminosuccinic acid) caused a loss of stimulating activity.

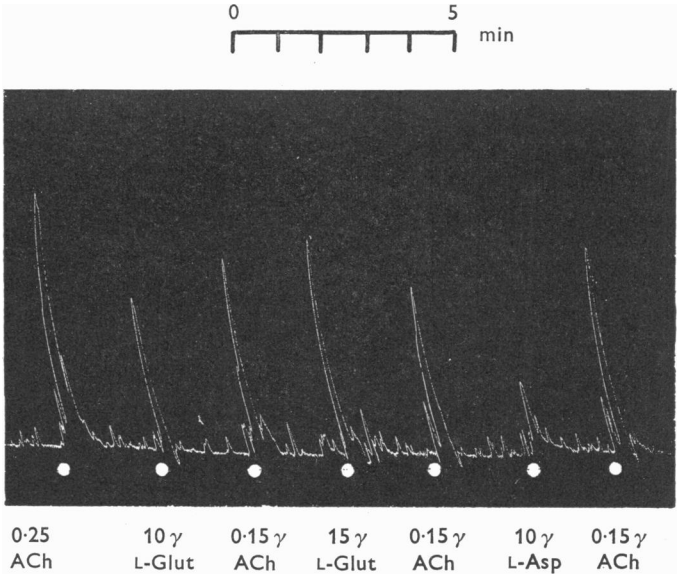

Fig. 1. Hind gut of *Astacus astacus* in 2·5 ml. bath of crayfish saline solution. Responses to acetylcholine (ACh), L-glutamic acid (L-Glut) and L-aspartic acid (L-Asp).

Introduction of further groups into the carbon chain. Introduction of a methylene group into L-glutamic acid at the γ-position halved the activity, but L-γ-methyleneglutamic acid was slightly more active than the γ-hydroxy derivative of this acid, which stimulated at a concentration of 2×10^{-5} g/ml. The β-hydroxy derivatives of DL-glutamic and DL-aspartic acids were also active at a concentration of 2×10^{-5} g/ml.

DISCUSSION

The observation that the hind gut of *Astacus astacus* is stimulated by acetylcholine, adrenaline, and noradrenaline is in accordance with the results of Florey (1954*a*, 1961), who used the species *Procambarus clarkii*, *Orconectes virilis*, and *Pacifastacus leniusculus*. But the finding that L-glutamic acid stimulated the hind gut differs from Florey's observation

TABLE 1. Action of glutamic acid and of structurally related compounds on the crayfish hind gut

Type of compound	Name	Chemical formula	Action* on crayfish hind gut	Threshold concentration for stimulation (g/ml.)
α-Amino dicarboxylic acids	L-Glutamic acid	$COOH\ CH(NH_2)\ (CH_2)_2COOH$	++	5×10^{-6}
	D-Glutamic acid	$COOH\ CH(NH_2)\ (CH_2)_2COOH$	+	4×10^{-5}
	L-Aspartic acid	$COOH\ CH(NH_2)CH_2COOH$	++	1×10^{-5}
	DL-α-Aminoadipic acid	$COOH\ CH(NH_2)\ (CH_2)_3COOH$	+	6×10^{-5}
	DL-α-Aminopimelic acid	$COOH\ CH(NH_2)\ (CH_2)_4COOH$	0	—
Amide derivatives	L-Glutamine	$COOH\ CH(NH_2)\ (CH_2)_2CONH_2$	0	—
	L-Asparagine	$COOH\ CH(NH_2)CH_2CONH_2$	0	—
	L-Glutamic acid diamide	$CONH_2CH(NH_2)\ (CH_2)_2CONH_2$	0	—
	L-Aspartic acid diamide	$CONH_2CH(NH_2)CH_2CONH_2$	0	—
β-Amino and diamino derivatives	β-Aminoglutaric acid	$COOH\ CH_2CH(NH_2)CH_2COOH$	0	—
	DL-α-γ-Diaminoglutaric acid	$COOH\ CH(NH_2)CH_2CH(NH_2)COOH$	0	—
	α-β-Diaminosuccinic acid	$COOH\ CH(NH_2)CH(NH_2)COOH$	0	—
γ-Methylene derivative	L-γ-Methyleneglutamic acid	$COOH\ CH(NH_2)CH_2CH{=}CH_2COOH$	++	1×10^{-5}
γ-and β-Hydroxy derivatives	L-Allo-γ-hydroxyglutamic acid	$COOH\ CH(NH_2)CH_2CHOHCOOH$	++	2×10^{-5}
	DL-Allo-β-hydroxyglutamic acid	$COOH\ CH(NH_2)CHOHCH_2COOH$	++	2×10^{-5}
	DL-Threo-β-hydroxyaspartic acid	$COOH\ CH(NH_2)CHOHCOOH$	++	2×10^{-5}
	DL-Erythro-β-hydroxyaspartic acid	$COOH\ CH(NH_2)CHOHCOOH$	++	2×10^{-5}

* ++ = stimulation; + = weak stimulation; 0 = no stimulation when tested at concentrations up to 10^{-4} g/ml.

that monosodium glutamate and glutamic acid had an inhibitory action on his preparations at a concentration which caused a stimulation in the present experiments. It is possible that species differences could account for this discrepancy.

Robbins (1958, 1959) and van Harreveld (1958) showed that L-glutamic acid caused a contraction of the opener muscle of the crayfish claw, although it was not possible to ascertain whether the acid was acting directly on the muscle. However, as Crustacea have striated muscle fibres only, it is possible that the intestinal and claw muscles have a similar pharmacology. The transience of the action of L-glutamic acid on the hind gut is also in accordance with observations of the effect of this acid on the claw muscle (Robbins, 1959; van Harreveld, 1960). This transient action together with resistance to the blocking action of acetylcholine antagonists, suggests that

L-glutamic acid does not stimulate by acting on the same intestinal structure as acetylcholine.

L-Glutamic acid (C_5) was found to be the most active compound of the range investigated, and from the results it can be concluded that the presence of two carboxyl groups and an α-amino group is required for stimulatory activity. The introduction of an additional hydroxyl or methylene group only slightly reduced the activity. These results are very close to those obtained by Curtis & Watkins (1960), who studied the structure–activity relationships of a similar range of compounds on the neurones in the mammalian spinal cord (with the exception of β-aminoglutaric acid, which stimulated in their experiments). Robbins (1959) found a similar correlation between the length of the carbon chain and the stimulatory activity of the α-aminodicarboxylic acids on the crayfish claw muscle. However, he found D-glutamic acid to be inactive.

The significance of the stimulation of crayfish muscles by L-glutamic acid is uncertain, and at present there is no further evidence to suggest that it is the excitatory transmitter at the neuromuscular junction. Experiments on the mammalian central nervous system (Curtis *et al.* 1960; Curtis & Koizumi, 1961) have shown that L-glutamic acid produces non-specific excitation of several different types of neurones; however, this does not necessarily indicate that the action on crayfish muscles is also non-specific.

SUMMARY

1. Acetylcholine, noradrenaline, and adrenaline caused contractions of the isolated hind gut of the crayfish *Astacus astacus* L. The action of acetylcholine was blocked by acetylcholine antagonists. Histamine, ATP and substance P were ineffective.

2. L-Glutamic acid also caused contractions of the preparation, but the effect was not blocked by acetylcholine antagonists.

3. D-Glutamic acid was 10 times less active than the L-isomer.

4. A range of compounds chemically related to L-glutamic acid have been tested for stimulating activity. L-Glutamic acid was the most active compound tested. It was found that the presence of two carboxyl groups and an α-amino group were essential for activity. The introduction of a hydroxyl or of a methylene group slightly reduced the activity.

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