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Quisqualamine, a novel γ-aminobutyric acid (GABA)-related depressant amino acid

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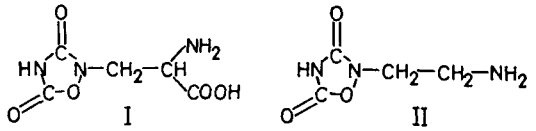
A new substance, quisqualamine, the decarboxylated analogue of quisqualic acid, predictably depressed electrical activity of neurons of the frog and rat spinal cord *in vitro* and of the mouse spinal cord *in vivo*. In the *in vitro* preparations, the action of quisqualamine was associated with a prolonged depolarization of primary afferent terminals which was sensitive to blockade by picrotoxin and bicuculline and which was also depressed by strychnine. This suggests an interaction of quisqualamine with presynaptic receptors for both GABA and β-alanine. Post-synaptic actions of quisqualamine, which were less marked than those at presynaptic sites, also appeared to be predominantly GABA-mimetic *in vitro*, though a sensitivity to the GABA-antagonist bicuculline could not be demonstrated *in vivo*.

Without known exception acidic amino acids that have excitatory actions in the mammalian and amphibian cns give rise to depressant amino acids on α-decarboxylation. Apart from the prototype pair, glutamate and γ-aminobutyric acid (GABA), other examples are aspartate and β-alanine, cysteate and taurine, homocysteate and 3-aminopropane sulphonic acid, and ibotenate and muscimol (Curtis & Watkins, 1960; Curtis, Phillis & Watkins, 1961; Johnston, Curtis & others, 1968). Hence the recent discovery of the potent excitatory action of quisqualic acid (I) (Shinozaki & Konishi, 1974; Biscoe, Evans & others, 1976) suggested that the corresponding decarboxylated compound (II) would probably be a depressant substance. We have now synthesized a



I II

compound, quisqualamine, believed to have this structure, and have investigated its actions on spinal motoneurons and primary afferent terminals of the frog and immature rat *in vitro* and also on mouse neurons *in vivo*.

MATERIALS AND METHODS

Preparation of quisqualamine

A solution of 1,2,4-oxadiazolidine-3,5-dione (Zinner & Stoffel, 1969) (230 mg, 2·3 mmol) and *N*-carbobenzyloxy-2-iodoethylamine (690 mg, 2·3 mmol) in a mixture of triethylamine (9 ml) and pyridine (23 ml) was refluxed for 1 h, then cooled and evaporated to dryness. The residue was shaken with HBr/acetic acid (40 ml) and left to stand at room temperature for 1·5 h. Evaporation of this mixture gave a red oil, which was shaken with water (10 ml) and centrifuged. The supernatant liquid was passed through a column of BioRad AG-1-X8 acetate (200–400 mesh, 10 ml), and the column was washed with water (6 × 25 ml). The first two water fractions were evaporated, and the residue redissolved in water (10 ml), and passed through a column of BioRad AG-50W-X8-NH_4^+ (200–400 mesh, 10 ml). The column was washed successively with water (20 × 10 ml), M pyridine (10 × 10 ml) and 2M NH_4OH (150 ml). Evaporation under reduced pressure of the latter eluate yielded quisqualamine as a white crystalline product (31 mg, 10% yield). It was recrystallized by the addition of 3 volumes of ethanol to an aqueous solution of the substance, followed by the dropwise addition of ether to incipient turbidity, yielding colourless prisms, m.p. 188–190°. Elemental analysis: Found, C 33·18, H 5·10, N 28·12%; $C_4H_7N_3O_3$ requires C 33·10, H 4·86, N 28·96%. On high voltage paper electrophoretograms the substance was cationic in buffer solutions of pH below 6 and anionic in buffers of pH above 8. Its mobility in buffers of pH 3–5 was similar to that of GABA, suggesting that the pKa of the oxadiazolidinedione moiety is close to that of the carboxyl group of GABA (pKa 4·03) (King, 1954). The pKa of the same ring system in quisqualic acid has been reported to be 4·15 (Takemoto, Takagi & others, 1975).

Isolated hemisected spinal cords of frogs (Biscoe & others, 1976) or rats (Otsuka & Konishi, 1974; Evans, 1978) were prepared for dorsal root stimul-

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ation and for dorsal and ventral root recording as previously described. Briefly, the potentials generated by electrical stimulation of a dorsal root (DR) or by superfusion of amino acids were recorded from the corresponding ventral root (VR) and a DR adjacent to the stimulated root. For this purpose the distal ends of the appropriate roots were placed in contact with non-polarizable (Ag/AgCl) recording electrodes, and potential changes measured between each of these electrodes and similar electrodes in contact with the superfusion medium. The roots were insulated from the medium with petroleum jelly. Upward deflection on the potential records indicates an increase in positivity of the distal recording electrode corresponding to depolarization of motoneurons (VR records) or primary afferent terminals (DR records). The standard superfusion fluids were as follows: frog (mM), NaCl, 111; KCl, 2; $CaCl_2$, 2; tris 10; glucose, 12; adjusted to pH 7·5 with HCl, temperature maintained at 12 ± 0·5°, flow rate 1·5 ml min^{-1}. Rat (mM), NaCl, 118; KCl, 3; $NaHCO_3$; 24; $CaCl_2$, 2·5; glucose, 12; gassed with 5% CO_2 in oxygen; pH 7·4; temperature 20 ± 0·5°; flow rate 0·8 ml min^{-1}. The continuous flow of these media was interrupted at regular intervals to allow the superfusion of 2 ml of medium containing quisqualamine or other amino acids. In some experiments, the media contained 10^{-7} M tetrodotoxin to abolish synaptically relayed effects and thus to allow direct effects of the substances on motoneurons or primary afferent terminals to be assessed.

Quisqualamine was also applied microelectrophoretically near mouse spinal interneurons using conventional techniques. Details of the surgical procedure have been described (Biscoe, Headley & others, 1977). The multi-barrelled micropipettes contained L-glutamate Na (0·2 M, pH 8), DL-homocysteate Na (0·2 M, pH 8), quisqualamine (50 mM, pH 3·5, HCl), GABA (0·5 M, pH 3·0, HCl), glycine (0·5 M, pH 3·5, HCl), bicuculline hydrochloride (10 mM in 165 mM NaCl, pH 4·0) and strychnine sulphate (10 mM in 165 mM NaCl, pH 5·0).

RESULTS

Frog spinal cord

Fig. 1 illustrates the effects of quisqualamine and GABA on the unblocked frog spinal cord. Quisqualamine (100–500 μM) depressed spontaneous VR potentials (VRPs) and VRPs evoked by dorsal root stimulation (DR-VRPs). These effects were associated with marked DR depolarization (which masked effects on DR-DRPs and on spontaneous DRPs)

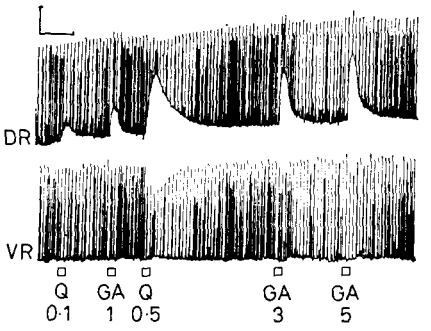

FIG. 1. Effect of GABA (GA) and quisqualamine (Q) on primary afferent fibres (DR) and motoneurons (VR) of frog isolated hemisected spinal cord. Spontaneous synaptic activity is seen superimposed on activity evoked by dorsal root stimulation (supramaximal pulses, 1 min^{-1}). Concentrations in mM shown beneath record. Calibration: vertical 1 mV; horizontal 10 min.

and with either a small VR hyperpolarization or with no overt change in VR polarity. GABA (1–5 mM) produced similar responses except that they were considerably more rapid in onset and offset than those of quisqualamine. On TTX-blocked spinal cords (Fig. 2A-C, control responses), the effects of the two depressants on DR and VR polarity were similar to those observed in unblocked cords. The potency of quisqualamine relative to GABA in such

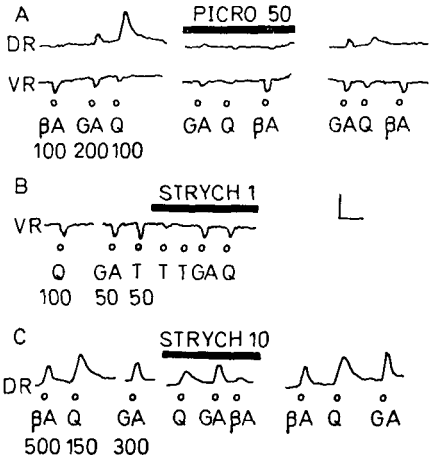

FIG. 2. Effect of picrotoxin (PICRO) and strychnine (STRYCH) on responses of frog motoneurons (VR) and primary afferent fibres (DR) evoked by β-alanine (βA), GABA (GA), quisqualamine (Q) and taurine (T). Agonist and antagonist concentrations shown in μM. The recordings in the presence of picrotoxin in A and in the presence of strychnine in C are shown 30 min after the entry of antagonist solutions. The recordings following picrotoxin and strychnine treatment in A and C respectively were obtained 12 h after washout of antagonist solutions. Calibration: vertical 0·5 mV; horizontal 10 min.

preparations was 3·2–10 for DR depolarizations (8 preparations) and 0·4–1·25 for VR hyperpolarization when these latter responses could be elicited (4 preparations).

In the frog spinal cord, receptors for depressant amino acids may be differentiated by the use of selective antagonists. Picrotoxin and bicuculline specifically depress VR hyperpolarizations produced by GABA, while similar VR responses to taurine and β-alanine are relatively unaffected by these agents. Conversely, strychnine specifically depresses VR hyperpolarizations produced by β-alanine and taurine without affecting GABA responses (Evans & Watkins, 1975). At somewhat higher concentrations than those necessary to block VR hyperpolarizations, strychnine also depresses DR depolarizations produced by β-alanine and taurine, without affecting DR responses to GABA; however, picrotoxin and bicuculline are both less specific against DR than against VR responses to the three depressant amino acids (Barker, Nicoll & Padjen, 1975). In the present experiments, picrotoxin (5–50 μM) markedly reduced or abolished DR depolarizations produced by quisqualamine or GABA, and depressed VR hyperpolarizations produced by these amino acids, while VR responses to β-alanine were unaffected by the antagonist (Fig. 2A). Similar results were obtained with bicuculline (5–50 μM). In contrast, strychnine (1 μM) had little effect on VR responses to quisqualamine or GABA, but abolished responses to taurine (Fig. 2B). In addition, strychnine (10 μM) reduced quisqualamine-induced DR depolarizations and almost abolished β-alanine-induced DR depolarizations while similar responses to GABA were unaffected by the alkaloid (Fig. 2C).

Rat spinal cord

GABA and related amino acids cause VR depolarization in the rat preparation rather than hyperpolarization as in the frog spinal cord (Evans, 1978). Also, GABA-induced DR depolarization, reflecting the depolarization of primary afferent terminals by the amino acid, is produced by much lower concentrations of GABA in the rat than in the frog. When tested on rat spinal cords (Fig. 3), quisqualamine depressed DR-VRP's and spontaneous VRP's such effects on DR-VRP's being weaker than those observed in the frog, while the effects on spontaneous activity were similar in the two species. In addition, quisqualamine depolarized both dorsal and ventral roots but was less potent than GABA on each root. Dorsal root responses to quisqualamine were of greater magnitude and duration than ventral root

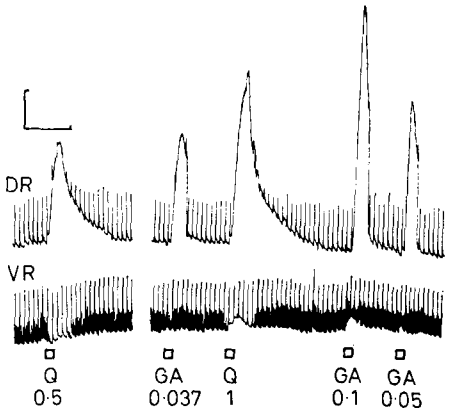

FIG. 3. Effect of GABA and quisqualamine on primary afferent terminals and motoneurons of immature rat hemisected spinal cord. Details as for Fig. 1.

responses. On TTX-blocked cords the range of relative potencies (quisqualamine to GABA) was 0·3–1·0 (n = 4) for DR depolarizations, and 0·08–0·3 (n = 3) for VR depolarizations. Quisqualamine-induced DR responses were abolished by bicuculline (25 μM) or picrotoxin (50 μM), and depressed by strychnine (10 μM). VR depolarizations induced by quisqualamine were also depressed by bicuculline (25 μM) or picrotoxin (50 μM); the effects of strychnine on these responses was not investigated.

Mouse spinal neurons

In microelectrophoretic experiments on L-glutamate- or DL-homocysteate-excited dorsal horn interneurons of the mouse spinal cord, quisqualamine had a weaker but more prolonged action than that of GABA. Based on equi-effective ejection currents, the potency of quisqualamine relative to that of GABA on 13 cells ranged between 0·02 and 1·2. This depressant action of quisqualamine appeared to be resistant to both bicuculline (9 cells) and strychnine (3 cells) with applications of these alkaloids sufficient to depress the actions of GABA and glycine respectively. An example of the action of quisqualamine, and its resistance to bicuculline, is shown in Fig. 4.

DISCUSSION

These results indicate that, *in vitro*, the post-synaptic effects of quisqualamine resemble those of GABA more than those of other inhibitory amino acids. The apparent resistance to bicuculline of the presumably postsynaptic action of quisqualamine on mouse spinal neurons *in vivo*, which contrasts with the potent antagonism by bicuculline and picrotoxin of

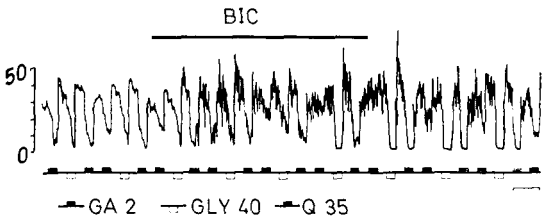

FIG. 4. Depression of firing of a mouse dorsal horn interneuron by microelectrophoretically administered GABA (GA), glycine (GLY) and quisqualamine (Q). The firing of this neuron was maintained by the continuous administration of L-glutamate (35 nA). Ejection currents and symbols for the depressants are shown beneath the record. The effects of GABA, but not those of quisqualamine or glycine, were antagonized by bicuculline (BIC) (25 nA) administered during the period indicated by the bar above the record. Calibration vertical: 50 spikes s^{-1}; horizontal: 1 min.

quisqualamine-induced responses in the isolated tissues, may be a consequence of different uptake rates of the two agonists. Thus, the more prolonged action of quisqualamine, compared with that of GABA, could be due to the lack of efficient uptake mechanisms for quisqualamine. In this case, quisqualamine might reach more distant receptors than GABA, such receptors possibly being out of range of bicuculline when the antagonist is applied at just-sufficient ejection currents to suppress the action of GABA.

The main feature of the action of quisqualamine was the prolonged depolarization of dorsal roots which it produced in the isolated frog and immature rat spinal cords. Though this action appeared to be predominantly GABA-mimetic, the fact that DR responses to quisqualamine were somewhat sensitive to strychnine, in addition to their marked sensitivity to picrotoxin and bicuculline, may indicate some participation of β-alanine and taurine receptors in the total presynaptic action of the substance.

The prolonged depolarizing action of quisqualamine on primary afferent terminals is not dissimilar from that exerted by pentobarbitone (Nicoll, 1975) or etomidate (Evans & Hill, 1977). It is thus possible that quisqualamine may be useful as a basis for the development of new central depressant drugs. Meanwhile, quisqualic acid and quisqualamine provide yet a further instance of the close structural relationship between excitatory and depressant amino acid molecules, and thus, most probably, between receptors for these two types of neuroactive amino acids.

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