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GABAergic Activity of Quisqualamine and Homoquisqualamine in Hemisected Spinal Cord *in vitro* Preparation

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α-Decarboxilation of excitatory amino acids can produce derivatives with depressant actions on the central nervous system. Examples are aspartate-B-alanine and glutamate-GABA. Quisqualate derivatives by α-decarboxilation, Quisqualamine (QUAM) and Homoquisqualamine (HOMOQUAM) (with an extra methylene group in the molecular chain), were studied in isolated spinal cord *in vitro* preparation. Dose-dependent depolarizations and inhibition of spontaneous ventral root potentials (sVRP) were induced by QUAM and HOMOQUAM in unblocked, Mg^{2+} free, hemisected cord. Ventral root evoked potentials by electrical stimulation of dorsal root (2ms, 30V, pulse/30 sec) (DR-VRP) remained unchanged. In Tetrodotoxin (TTX) medium, HOMOQUAM showed a twofold increment of relative potency respect to QUAM depolarizations. Actions evoked by QUAM and HOMOQUAM were not affected by the addition of N-Methyl-D-Aspartate (NMDA) receptor blockers $Mg^{2+}$and DL-AP5; non-NMDA antagonist CNQX and GABA B receptor antagonist 2-hydroxysaclofen. In presence of GABA A receptor blockers Bicuculline MeCl or Picrotoxin, the actions evoked by QUAM and HOMOQUAM were blocked. The results obtained show that GABA A receptor seemed to mediate QUAM and HOMOQUAM activity in spinal cord *in vitro* preparation. The addition of a methylene group in the molecular chain increased the potency twice although the kinetic of the drug did not appeared to have changed. The development of new compounds with depressant activity in the central nervous system may be useful in assessing the physiological and therapeutic significance of central GABA receptors, especially if the blockade of spontaneous activity is not followed by an alteration of the neuronal integration and synaptic transmission reflected in the DR-VRP.

Key words: Spinal cord, *in vitro* preparation, NMDA, nonNMDA, GABA A, GABA B, Quisqualamine, Homoquisqualamine, DR-VRP, TTX.

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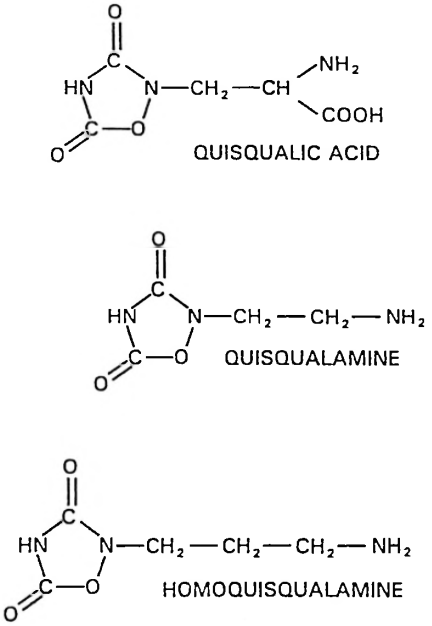
The α-decarboxilation of amino acids with excitatory activity (EAA) upon the central nervous system of mammals and amphibians can produce substances with depressant properties. Some well known examples of this phenomenon are the pairs aspartate and its derivative β-alanine, ibotenate and muscimol, and glutamate and gamma-amino-butyric acid (GABA) (4). Quisqualamine, the product of α-decarboxilation of the EAA quisqualate, is one more of those examples, that posses the ability to bind a GABA receptor (8).

The effects of both quisqualamine (QUAM) and its derivative homoquisqualamine (HOMOQUAM) (see schema) have been studied in the isolated immature spinal cord *in vitro* preparation. The aims of these experiments have been, first of all, to analyse whether the addition of a methylene group within the molecular chain of QUAM induces any change in the actions evoked by this substance in the neuronal activity of isolated spinal cord of immature rats. Secondly, to study whether ventral root evoked potentials, driven by electrical stimulation of dorsal root, as well as spontaneous activity, are altered by the administration of these compounds. Finally, the specificity of their actions upon NMDA, non-NMDA, GABA A and GABA B receptors has been studied.



Structure of quisqualic acid, quisqualamine and homoquisqualamine.
α-decarboxilation of quisqualic acid leads to quisqualamine from which homoquisqualamine is synthesized by means of the inclusion of a methylene group within the molecular chain.

Materials and Methods

Spinal cords from immature rats were prepared for recording evoked potentials following the technique described previously in detail (1, 5, 15).

Very briefly, 2 to 5 day old Wistar rats were anaesthetised with urethane i.p. (2.5 g/kg) and, after decapitation, the spinal cord was dissected out and placed in a dissecting bath at room temperature in Ringer's solution. After isolation of roots and longitudinal hemisection, the hemicord was placed on a warm water controlled chamber at a constant temperature of 25 °C. One lumbar dorsal root (L1 to L4) was placed on a stimulating Ag/AgCl electrode, whereas its correspondent ventral root was situated on a Ag/AgCl-Agar electrode by which evoked activity was recorded. Both electrodes and roots were insulated from the cord by means of a grease gap. A third electrode was used to earth the superfusion medium (7).

The hemicord was superfused continuously (2 ml/min) with Ringer's solution gassed with O_2 (95 %) and CO_2 (5%) at a temperature of 25 °C and a final pH of 7.4. The composition of the solution was (mM): NaCl 118, $NaHCO_3$ 25, KCl 3, $CaCl_2$ 2.5, Glucose 12. Drugs studied in this system were dissolved in Ringer's and applied by superfusion in 5 ml aliquots. Ringer's fluid flow was interrupted while the drug was applied.

The preparation was stimulated with 0.2 ms and 30 V pulses, twice per minute. Dorsal root-ventral root evoked potentials (DR-VRP) were continuously recorded (fig. 1) as a baseline where upward deflections indicate an increase of positivity of the recording electrode and thus, a depolarization of the cord. Single DR-VRP were also recorded during the application of a drug, and their parameters compared to the pre- and post-drug potentials. Ventral root spontaneous potentials (sVRP) can also be seen in this kind of recording (fig. 1). In all experiments the hemicord was resting for at least one hour before a drug was applied.

In a second group of experiments QUAM and HOMOQUAM were studied in presence of tetrodotoxin (TTX), 10 µM initial dose and 0.1 µM continuously in normal Ringer's solution. In these experiments drugs were applied in 2 ml aliquots and no electrical stimulation was applied. Potency of depolarization and responses against EAA and GABA receptor antagonists were tested in these conditions. The drugs used were: Quisqualamine, Homoquisqualamine, N-methyl-D-aspartate, Quisqualate, AMPA (α–amino-3-hydroxy-5-methylisoxazole-4-propionic acid), Kainate, $MgSO_4.7H_2O$, DL-AP5 (2-amino-5-phosphonopentanoate), CNQX (6-cyano-7-nitroquinoxaline 2,3 dione), Bicuculline methochloride, Baclofen, 2-Hydroxy-saclofen, Picrotoxin and Tetrodotoxin. All studies were performed on at least five hemicords.

All drugs except picrotoxin and tetrodotoxin (Sigma) were obtained from Tocris Neuramin.

Preliminary data have been published elsewhere (2).

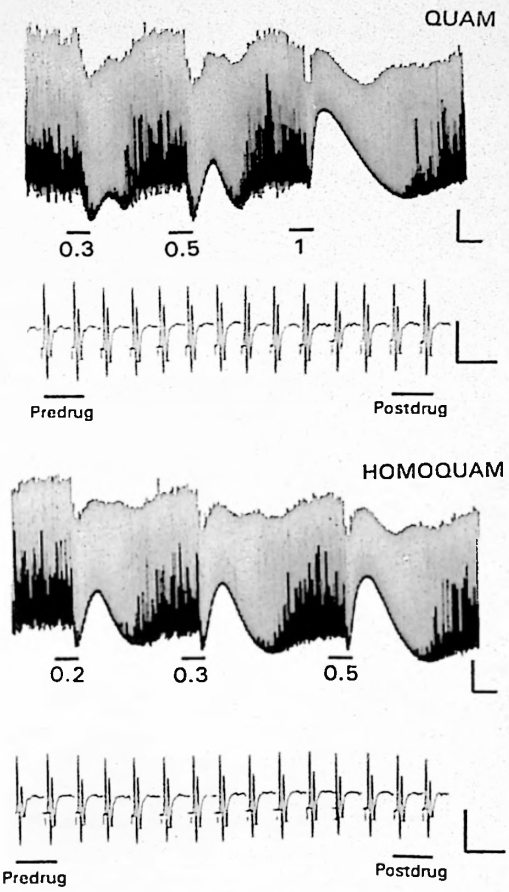


Fig. 1. *Effects produced by QUAM and HOMOQUAM in unblocked immature spinal cord recordings.* Both QUAM and HOMOQUAM produced a dose-dependent depolarization of the preparation but DR-VRP remained unchanged with all doses tested. Note how the spontaneous activity was totally inhibited after the administration of the compounds. Scale bars: horizontal: 5 min for cord recordings and 10 ms for DR-VRP; vertical: 1 mV for cord recordings and 10 mV for DR-VRP.

Results

Figure 1 reproduces some original recordings obtained from ventral root, after electrical stimulation of the correspondent dorsal root, in unblocked cord (normal Ringer medium). Deflections of the baseline were always obtained after the application of QUAM and HOMOQUAM 0.2 to 1mM. Both substances induced a dose-dependent depolarization of the system that was quantified in TTX medium. In all cases, a total inhibition of the spontaneous activity recorded in ven-

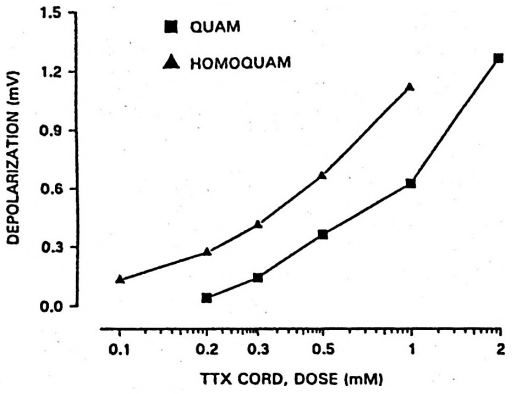


Fig. 2. *Dose-response curves obtained with QUAM and HOMOQUAM in Ringer-TTX medium (10 μM initial dose and 0.1 μM continuously).* HOMOQUAM showed to be twofold more potent than QUAM and curves were parallel.

tral root was observed immediately after the administration of QUAM and HOMOQUAM; the effect lasted an average of 13 minutes and recovered completely with no significant rebound. All parts of DR-VRPs (fast and slow components, positive and negative waves) remained unchanged with all doses during the depolarizations, when compared to pre-drug activity.

In TTX medium, similar depolarizations were obtained after the administration of QUAM and HOMOQUAM; the effect was dose dependent and dose-response curves were parallel (fig. 2). HOMOQUAM showed at all doses a twofold increase of potency in relation to QUAM (0.62±0.07 mV QUAM 1 mM and 0.66±0.09 mV HOMOQUAM 0.5 mM, n=7, mean and s.e.m.), (figs. 3 and 4).

To see whether NMDA receptors mediated some of these actions NMDA, Quisqualate, QUAM and HOMOQUAM were tested both in normal TTX medium and in TTX plus Mg^{2+} ($MgSO_4.7H_20$, 1 mM) (NMDA channel blocker), and DL-AP5 (50 μM) (NMDA receptor competitive antagonist) (fig. 3 B).

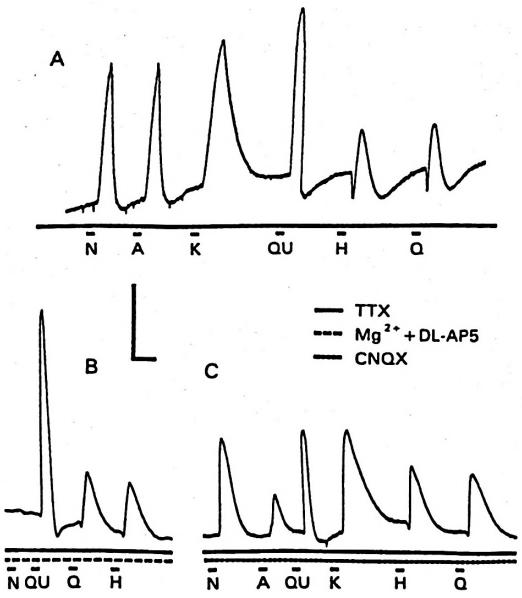


Fig. 3. *Activity of QUAM and HOMOQUAM tested against NMDA and nonNMDA receptor antagonists.*
A) Depolarizations obtained in TTX-blocked spinal cord after the administration of NMDA (N) 7 μM, AMPA (A) 2 μM, Kainate (K) 5 μM, Quisqualate (QU) 3 μM, HOMOQUAM (H) 0.5 mM and QUAM (Q) 1 mM. B) During the continuous infusion of NMDA-blockers, Mg^{2+} ($MgSO_4.7H_2O$, 1 mM, NMDA channel blocker), and DL-AP5 (50 μM, NMDA receptor competitive antagonist), response evoked by NMDA was totally abolished whereas QUAM- and HOMOQUAM-activity was not modified. C) CNQX (2 μM, non-NMDA unspecific antagonist) did not either change those responses, however, AMPA, QUISQUALIC, KAINATE and NMDA were partially depressed. Scale bars: horizontal: 5 min; vertical: 1 mV.

Effects evoked by QUAM and HOMOQUAM remained unchanged in potency and duration under these conditions.

Activity evoked by NMDA (7 μM) was totally inhibited whereas depolarisations induced by Quisqualate (3 μM) did not change in any significant way.

Non-NMDA receptors involvement in QUAM and HOMOQUAM actions

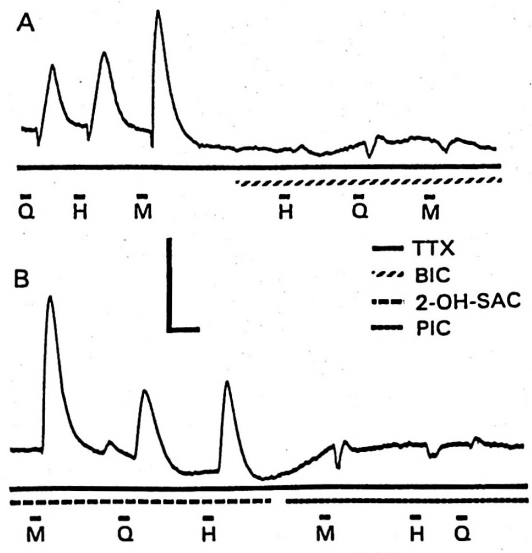


Fig. 4. *Activity of QUAM and HOMOQUAM tested against GABA A and B receptor antagonists.* A. The activity evoked by QUAM (Q), HOMOQUAM (H) and Muscimol (M), in TTX-blocked spinal cord, was blocked by Bicuculline (BIC, Bicuculline MeCl 20 μM, GABA A antagonist). B. The GABA-B blocker, 2-hydroxysaclofen (0.5 mM, 2-OH-SAC), did not modify the response, whereas Picrotoxin (PIC, 50 μM, GABA A Chloride channel blocker) produced similar inhibition to Bicuculline (Doses and scale bars as in fig. 3).

were studied next (figure 3 C). In these experiments, depolarizing activity induced by AMPA (2.5 μM), quisqualate (3 μM), Kainate (5 μM) and NMDA (7 μM) were partially but significantly inhibited after the blockade of non-NMDA receptors by the unspecific antagonist CNQX (2 μM). However, depolarizations obtained after the administration of QUAM and HOMOQUAM remained unchanged.

Figure 4 shows experiments performed in TTX medium to study the possible mediation of GABA receptors in actions evoked by QUAM and HOMOQUAM When GABA A antagonist Bicuculline MeCl (20 μM) was applied continuously onto the spinal cord, depolarizations evoked by QUAM , HOMOQUAM and Muscimol (10 μM), (GABA A agonist) were completely blocked (fig. 4 A). Similar results were obtained with Picrotoxin (50 μM, GABA A Chloride channel blocker) (fig. 4 B). However, when GABA B receptors were blocked by means of 2-hydroxysaclofen (0.5 mM, GABA B antagonist) Muscimol-, QUAM- and HOMOQUAM-evoked activity resulted unaltered.

Discussion

Results obtained in these experiments point towards an *in vitro* activity induced by QUAM and HOMOQUAM more similar to that obtained with muscimol than to quisqualate. NMDA and NMDA receptors do not seem to be involved in those actions, at least in this *in vitro* spinal cord preparation, since the blockade of those receptors with Mg^{2+}, DL-AP5 and CNQX (11) produced no change in the activity induced by QUAM and HOMOQUAM. Moreover, it has been shown that the early monosynaptic component in the DR-VRP is selectively reduced by non-NMDA antagonists (9, 12), whereas the late longer latency or polisynaptic component is strongly attenuated by selective NMDA antagonists (6). The fact that the DR-VRP was not affected at all by QUAM and HOMOQUAM is an early argument for an improbable mediation of either NMDA or non-NMDA receptors in their actions.

On the other hand, the total inhibition of the activity induced by the GABA A competitive antagonist Bicuculline MeCl (3) and Picrotoxin, (as a blocker of GABA-induced Cl^- conductance responses) (3, 14) indicates a more than probable interaction upon this receptor. This action seems to be rather specific and the involvement of GABA B receptor appears to be very unlikely since no modification of the effects was observed when QUAM and HOMOQUAM were applied during the ad-

ministration of the GABA B antagonist 2-Hydroxysaclofen (10).

The inhibition of spontaneous activity that appeared after the administration of QUAM and HOMOQUAM in unblocked spinal cord is a characteristic of drugs that depress the activity of the central nervous system. It might indicate an involvement of these substances, and thus the GABA A receptor, in the generation of spontaneous activity within the spinal cord of mammals. In fact, the convulsant properties of bicuculline and picrotoxin are well known (3, 14), as well as the anticonvulsant (17) properties of some GABA A agonists. The therapeutic possibilities of such compounds are therefore encouraging, essentially in the treatment of models of neuronal overexcitation, and especially if processes of neuronal integration and generation of synaptic events are not modified as it appears with QUAM and HOMOQUAM.

The presence of a methylene group in the molecular chain of HOMOQUAM appeared to be responsible for a twofold increase of the potency of depolarization. The lasting of the effect, however, did not change, nor did the behaviour against the antagonists tested and the slope of the dose-response curve. All these facts may be indicative that, although the molecular change induced a significant increase in the potency of the compound, the kinetics did not appear to be modified in any way. That was not the case when quisqualate and homoquisqualate were tested as agonists for the glutamate metabotropic receptors (13). In this case, while quisqualate showed a rather low affinity, its derivative homoquisqualate behaved as a full potent agonist (16).

In conclusion, the α-decarboxilation products of quisqualate, QUAM and HOMOQUAM, produced GABA A mediated and dose-dependent depolarizations in spinal cord *in vitro* preparation. The presence of a methylene group in the molecule increased twofold that activity. sVRP were inhibited at all doses tested, whereas DRVRP remained unchanged. No involvement of NMDA and non-NMDA receptors were observed.

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La α-descarboxilación de aminoácidos excitatorios puede dar lugar a la aparición de compuestos con acciones depresoras sobre el sistema nervioso central, como los pares aspartato-β-alanina y glutamato-GABA. Se estudian en la preparación *in vitro* de hemimedula aislada los derivados por α-descarboxilación del quiscualato, la quiscualamina (QUAM) y la homoquiscualamina (HOMOQUAM), con un grupo metileno adicional en la cadena molecular, que inducen despolarizaciones de carácter dosis dependiente e inhiben los potenciales espontáneos de la raíz dorsal (DRP) en hemimedula no bloqueada y libre de Mg^{2+}. No se modifican los potenciales evocados en raíz ventral tras estimulación eléctrica de raíz dorsal (2 ms, 30 V, 1 pulso/30 s) (DR-VRP). En medio con tetrodotoxina (TTX), el HOMOQUAM evoca una potencia relativa dos veces mayor que QUAM, con curvas dosis-respuesta paralelas. Esta actividad no se modifica tras la administración de bloqueantes del receptor N-metil-D-aspartato (NMDA): Mg^{2+} y DL-AP5; no-NMDA: CNQX, y GABA B: 2-hidroxisaclofén. En presencia de bicuculina y picrotoxina, bloqueantes GABAérgicos A, las acciones evocadas por QUAM y HOMOQUAM quedan abolidas. Los resultados obtenidos muestran que el receptor GABA A parece ser el mediador de la actividad evocada por QUAM y HOMOQUAM en la preparación *in vitro* de medula aislada. La adición de un grupo metileno a la cadena molecular incre-

menta la potencia despolarizante en dos veces, aunque la cinética del compuesto no parece haber cambiado. Este tipo de compuestos podría ser eficaz en la remisión de la hiperexcitabilidad neuronal porque, aunque se inhibe la actividad espontánea, los sistemas de integración neuronal y transmisión sináptica reflejados en el DR-VRP no se modifican.

Palabras clave: Medula espinal, Preparación *in vitro*, NMDA, no-NMDA, GABA A, GABA B, Quiscualamina, Homoquiscualamina, DR-VRP, TTX.

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