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Blockade of excitatory synaptic transmission by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the hippocampus in vitro

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Superfusion of hippocampal slices with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 2–5 μM) reversibly blocked the Schaffer collateral and mossy fibre excitatory postsynaptic potential (EPSP), while sparing the fast and slow γ-aminobutyric acid (GABA)-mediated inhibition. Membrane potential, input resistance and spike accommodation were not altered. Inward currents induced by quisqualate were reduced to a greater extent by CNQX than those induced by kainate or *N*-methyl-D-aspartate. We suggest that CNQX may be a useful antagonist to study excitatory amino acid-mediated synaptic transmission.

There is widespread interest in the excitatory amino acids and the transmissive processes they subserve. The availability of reasonably selective agonists for the three so far identified receptor subtypes (*N*-methyl-D-aspartate (NMDA), kainate and quisqualate) [5] along with selective antagonists for the NMDA receptor [8, 9] has allowed some functional investigation of the pharmacology and physiology of these receptors. Recently, it has been reported that 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) has a high affinity for quisqualate binding sites versus NMDA and kainate sites [4]. In the hippocampus, excitatory synaptic transmission in both the CA1 and CA3 regions are thought to be mediated by amino acids acting on non-NMDA receptors [1, 2]. We therefore investigated the action of CNQX on synaptic transmission and excitatory amino acid induced currents in the hippocampal slice preparation.

Male Wistar rats (90–125 g) were used. Transverse slices of hippocampus (500 μm) were cut and transferred to a submerged type recording chamber as described previously [6]. Briefly the slices were superfused (2 ml/min) at a temperature of 33–34°C. The artificial cerebrospinal fluid (ACSF) consisted of (mM): NaCl 126, KCl 3.5, $MgCl_2$ 1.3, NaH_2PO_4 1.2, $CaCl_2$ 2, $NaHCO_3$ 25 and glucose 11. The ACSF was

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gassed with 95% O_2/5% CO_2 and gave a pH of 7.3. The methods for stimulation, extracellular and intracellular recording have been described elsewhere [6, 7]. CA3 neurones were voltage-clamped using a single electrode voltage clamp amplifier (Axoclamp II). The sampling frequency was 3–4 kHz, 30% duty cycle. To ensure correct operation of the clamp, the voltage at the head stage amplifier was monitored on a separate oscilloscope. Microelectrodes filled with 3M CsCl were used for the voltage clamp experiments in order to reduce the potassium currents and improve the space clamp. Tetrodotoxin (TTX, 1 μM) was used to block the fast Na^+ transients. Drugs used were: D((−)-2-amino-5-phosphonopentanoic acid (AP-5; CRB); D(−)-2-amino-7-phosphonoheptanoic acid (AP-7; CRB); CNQX (Ferrosan); kainate (Sigma); NMDA (CRB); quisqualate (CRB); TTX (Sigma). Results are based on data from 26 neurons with membrane potentials greater than −56 mV with action potentials over 75 mV in amplitude. Twenty-three slices were used.

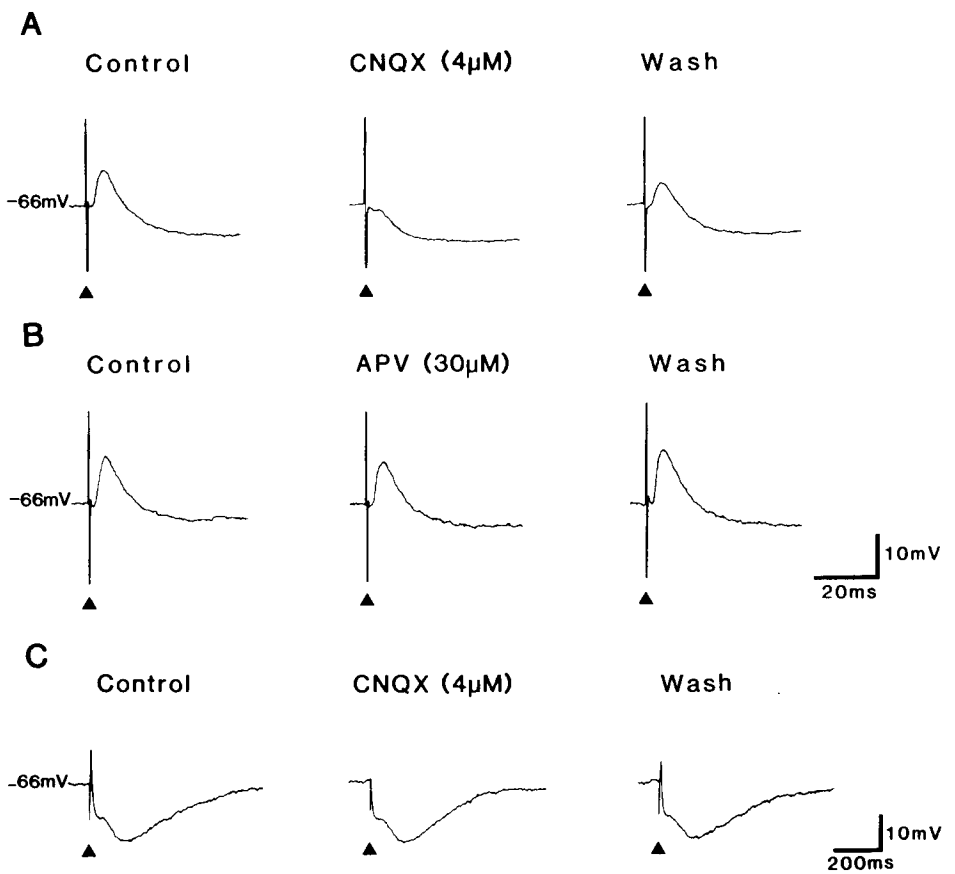

Fig. 1. CNQX but not the NMDA receptor antagonist, APV, blocks the mossy fibre EPSP. A: the EPSP evoked by hilar stimulation (triangles) was reversibly blocked by 4 μM CNQX, whereas it was not reduced by APV (B). C: the amplitude of the fast and slow IPSP was not reduced by CNQX (4 μM). Note the different time axis between A, B and C. All traces from the same cell.

Application of 2–5 μM CNQX ($n=6$) completely and reversibly blocked the mossy fibre excitatory postsynaptic potential (EPSP) (Fig. 1A). The blockade was very rapid taking approximately 2 min with 4 μM CNQX. Up to 30 min of wash was necessary for complete recovery. In contrast to CNQX, application of the NMDA receptor antagonists, AP-5 or AP-7 (50 μM), failed to reduce the EPSP (Fig. 1B) confirming

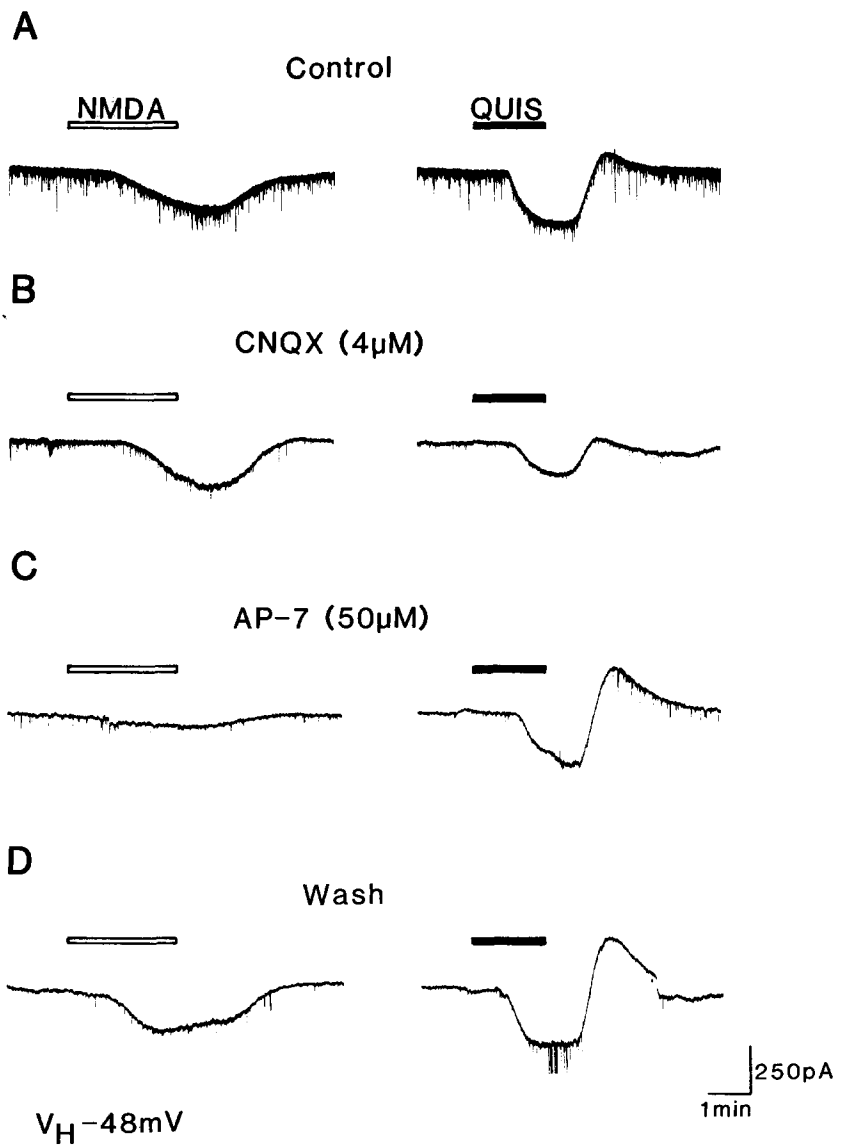

Fig. 2. Selective blockade of quisqualate and NMDA currents by CNQX and AP-7 respectively. Superfusion of CNQX reduced the current induced by quisqualate without altering the current induced by NMDA (B). Bath application of the NMDA antagonist AP-7 reduced the NMDA but not the quisqualate current (C).

previous observations [2]. CNQX (2–4 μM) also reversibly blocked the intracellular and extracellular EPSP ($n=4$) evoked in the CA1 region by Shaffer collateral stimulation without reducing the amplitude of the afferent volley (not shown).

CNQX abolished synaptic excitation without altering the synaptically mediated GABA inhibition in the CA3 region (Fig. 1C). The amplitude of the fast and slow inhibitory postsynaptic potentials (IPSP) were respectively $103 \pm 4\%$ ($\bar{x} \pm$ S.E.M.) and $105 \pm 3\%$ of control in 5 μM CNQX ($n=4$). This observation suggests that feedforward inhibition [3] results from the activation of a monosynaptic pathway.

The reduction in the EPSP by CNQX was not associated with changes in membrane potential, membrane input resistance or spike accommodation ($n=6$).

The effects of CNQX were also tested on the currents induced by kainate, NMDA and quisqualate to determine the selectivity of the antagonist. As shown in Fig. 2, CNQX had a greater effect on the currents induced by quisqualate than those induced by NMDA. These effects were time and dose dependent. Thus, the current induced by quisqualate (10 μM) was reduced to $56 \pm 17\%$ ($n=7$) and $32 \pm 4\%$ ($n=6$) of control by 4 μM and 10 μM of CNQX, respectively. The corresponding values for 20 μM NMDA were $77 \pm 14\%$ ($n=5$) and $66 \pm 11\%$ ($n=4$) of control for 5 min exposure to CNQX. AP-7 or AP-5 (50 μM, $n=4$) reduced the NMDA-induced currents but not those induced by quisqualate (Fig. 2) or kainate. The inward currents induced by kainate (200 nM) were slightly reduced by 10 μM CNQX ($78 \pm 17\%$ of control, $n=4$). Full recovery was observed within 1 h of washing CNQX.

From our observations, we suggest that CNQX is a potent antagonist of the Schaffer collateral and mossy fibre EPSPs. Moreover, CNQX appears to be a good antagonist of the quisqualate response. The order of potency against the excitatory amino acid agonists being quisqualate > kainate = NMDA. This suggests that the transmitter for the EPSP evoked by the Schaffer collateral and mossy fibre stimulation acts on the quisqualate type receptor. The potency, selectivity and reversibility make CNQX an attractive amino acid antagonist in the study of excitatory amino acids in synaptic transmission.

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