Effects of antagonists on quisqualate and nicotinic receptor-mediated currents of midbrain neurones in culture

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1 The action of non-N-methyl-D-aspartate (non-NMDA) and nicotinic antagonists on excitatory post-synaptic currents (e.p.s.cs) and on quisqualate (Quis)- and nicotine-gated currents was studied by use of whole-cell recording in dissociated culture of the rat midbrain.

2 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX; 0.1 μ M) and kynurenic acid (0.1 mM) attenuated network-generated and miniature e.p.s.cs while mecamylamine (100 μ M) and hexamethonium (400 μ M) had no effect. Acetylcholine (ACh) enhanced or suppressed e.p.s.cs. The suppressing effect of ACh was blocked by atropine (0.1–10 μ M).

3 ACh (50–1000 μ M) and quisqualate (Quis, 0.1–20 μ M) induced inward currents with the same reversal potential as e.p.s.cs.

4 Application of Quis and α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) in a low concentration (0.5 and 5 μ M, respectively) evoked a maintained current which was attenuated by CNQX (1 μ M) and kynurenic acid (0.5 mM) but not by mecamylamine (100 μ M).

5 Higher concentrations of Quis (5–20 μ M) and AMPA (50–100 μ M) evoked a transient and a maintained current component. Kynurenic acid (1 mM) reduced the transient but not the maintained component. CNQX (5–10 μ M) increased the maintained component without reducing the transient one; 20 μ M CNQX reduced both components.

6 ACh-induced transient current was mimicked by nicotine and reversibly and dose-dependently blocked by mecamylamine. Atropine (10 μ M), hexamethonium (400 μ M) as well as CNQX (100 μ M) and kynurenic acid (1 mM) did not affect the current.

7 Hexamethonium (50–400 μ M) voltage-dependently depressed the maintained current elicited by both Quis and ACh.

8 In conclusion, although the antagonists examined here seem to discriminate between non-NMDA and nicotinic receptor-mediated e.p.s.cs, they vary considerably in respect of their mode of action when tested on Quis, AMPA and ACh-induced currents.

Introduction

Both nicotinic and non-N-methyl-D-aspartate (non-NMDA) receptors have been shown to mediate fast synaptic excitation in the mammalian CNS (for a review of the literature see Nicoll *et al.*, 1990). Identity of action of excitatory amino acids (EAA) and the endogenously released transmitter and their pharmacological identity, i.e. the selective inhibitory effect of antagonists on both synaptic and exogenously induced events, had led to the conclusion that fast excitatory postsynaptic potentials (e.p.s.ps) in the mammalian CNS are generated by EAA. Recently a growing body of experimental evidence has pointed to the presence of functional nicotinic receptors in the brain (Egan & North, 1986; Lipton *et al.*, 1987; McCormick & Prince, 1987; Vidal & Changeux, 1989). Since nicotinic and non-NMDA agonists elicit an identical conductance change, it is important to determine the extent to which the selectivity of non-NMDA and nicotinic receptor antagonists pertains to the appropriate receptor type. To do this, we investigated the selectivity of the two competitive EAA antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Honoré *et al.*, 1988) and kynurenic acid (Perkins & Stone, 1982; Ganong *et al.*, 1983) and the two nicotinic receptor antagonists, mecamylamine and hexamethonium. The antagonists were tested for acetylcholine (ACh)- and quisqualate (Quis)-induced currents and spontaneous synaptic currents in midbrain cultures by use of whole-cell recording. Midbrain neurones have been shown to

express functional nicotinic receptors (Clarke *et al.*, 1985; Calabresi *et al.*, 1989), and *in situ* hybridization has revealed several genes of the nicotinic ACh receptor in this area (Goldman *et al.*, 1986; 1987).

Methods

Cell culture

Ventral tegmental tissue from 14 day old rat embryos was mechanically dissociated and plated on a primary culture of glial cells from the same area (Misgeld & Swandulla, 1989; Swandulla & Misgeld, 1990). Cells were cultivated in Eagles basal medium (BME, Gibco) supplemented with 10% heat-inactivated horse serum (Gibco), 0.3% glucose, 2 mM glutamine, penicillin (25 iu ml⁻¹, Sigma) and streptomycin (25 μ g ml⁻¹, Sigma). After 3 days in culture, horse serum was reduced to 2% and 10 μ M cytosine arabinoside (Ara-C Sigma) was added for 68 h to inhibit growth of mitotic cells.

Recording conditions

The whole-cell configuration (Hamill *et al.*, 1981) was used to record with a single electrode- (for specification of the clamp see Misgeld *et al.*, 1989) or a EPC-7-patch clamp amplifier. All currents were recorded at –80 mV holding potential unless otherwise stated. During electrophysiological experiments cells were bathed in a solution containing (in mM): NaCl 145, KCl 5, CaCl₂ 5, MgCl₂ 2, glucose 15, HEPES 10 at pH 7.3 and room temperature. Tetrodotoxin (TTX, 0.3 μ M) and picrotoxin (20 μ M) were added to the extracellular solution except while recording spontaneous synaptic potentials. Recording

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electrodes (3–5 M Ω), pulled from borosilicate glass (Clark, UK), were filled with (in mM): NaCl 10, CsCl 100, EGTA 5, CaCl₂ 0.25, glucose 10, HEPES 10 (pH 7.3). KCl was used instead of CsCl while recording synaptic activity. In some experiments K-gluconate replaced KCl in the intracellular solution. Data were stored on a chart recorder and on a PC.

Drugs were dissolved in extracellular solution (pH 7.3) and applied by superfusion from a wide bore (tip diameter 100 μ m) 3-barrelled pipette positioned 100–200 μ m from the recorded cell (Carbone & Lux, 1987). By this method, a quickly inactivating response to Quis application can be seen (Tang *et al.*, 1989) which is not detected if slower perfusion systems were used. Between drug applications, the cell was continuously washed with extracellular solution. Antagonists were tested by comparison with the control response before and after simultaneous application of agonist and antagonist. Results are expressed as mean $\pm$ s.e.mean with *n* equal to the number of cells studied.

Drugs

The following drugs were used: acetylcholine chloride (ACh), hexamethonium chloride, kynurenic acid, mecamylamine hydrochloride, picrotoxin, quisqualic acid (Quis), tetrodotoxin (TTX), all obtained from Sigma, F.R.G. α -Amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), D- α -amino-5-phosphonopentanoic acid (APV), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were from Tocris, England; nicotine salicylate was from Roth, F.R.G.

Results

Spontaneous synaptic activity

Cells were recorded between 3 and 8 weeks after plating. They exhibited spontaneous synaptic activity which was reduced by low Ca- (1 mM) and high Mg- (6 mM) containing extracellular solution (Figure 1a) and by TTX (0.3 μ M). Some synaptic potentials were reversed around -10 mV, when CsCl electrodes were used, and around -60 to -50 mV while recording with gluconate filled electrodes. The reversal potential of these synaptic events, the shift of their reversal potential with changes in intracellular Cl concentration and their sensitivity to picrotoxin (20 μ M) (Figure 1b) suggest that they are due to Cl conductance increase following the activation of GABA_A receptors.

In the presence of picrotoxin, synaptic activity shifted from irregular activity to rhythmically occurring clusters of e.p.s.ps (Figure 1b). These e.p.s.ps reversed at potentials positive to $+5$ mV and were attenuated by the excitatory amino acid receptor antagonists CNQX (1 μ M; Figure 1c,d) and kynurenic acid (0.5 mM; Figure 1c), while they were not reduced by α -amino-5-phosphonopentanoic acid (APV, 20–50 μ M; *n* = 4) in a voltage range between -80 and -20 mV. This suggests the generation of e.p.s.ps through a non-NMDA receptor type. In the presence of picrotoxin (20 μ M) and TTX (0.3 μ M), under voltage clamp, spontaneous miniature e.p.s.cs (Jarolimek & Misgeld, 1991) were observed (Figure 2b(ii)). They also reversed at holding potentials positive to $+5$ mV. Miniature e.p.s.cs were reduced by kynurenic acid (0.1–1.0 mM; *n* = 11; Figure 2a) and CNQX (0.1–10 μ M; *n* = 11; Figure 2b), but were neither affected by the nicotinic receptor antagonists mecamylamine (up to 100 μ M) and hexamethonium (up to 400 μ M) nor by the muscarinic antagonist atropine (1 μ M). ACh altered excitatory synaptic activity (in the presence of picrotoxin), even in cells in which it did not induce the inward current described below. In cells, in which a barrage of synaptic potentials followed the application of ACh (Figure 3a(i), b(ii)), atropine was ineffective. However, atropine (0.1–10 μ M) reversed the effect of ACh in cells in which ACh reduced network or miniature e.p.s.cs (Figure 3: b(ii), (iii); c(i), (ii)).

Figure 1 Synaptic activity in the midbrain culture. (a) Spontaneous network activity was reduced by low Ca- (1 mM), high Mg- (6 mM) solution. (b) Effect of picrotoxin (20 μ M) on spontaneous activity in the same neurone as in (a). $V_H = -70$ mV. Calibration in (a) applies also to (b). (c) Network activity in the presence of picrotoxin under voltage clamp and its reduction by kynurenic acid (Kyn 0.5 mM) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 1 μ M). (d) Effect of 1 μ M CNQX on e.p.s.p. clusters occurring in the presence of picrotoxin. Drug application periods in this and subsequent figures are indicated by horizontal bars. In current clamp recordings, spikes truncated by the frequency characteristics of the chart recorder. Under voltage clamp, holding potential was -80 mV in this and all other figures, unless stated otherwise.

Acetylcholine- and quisqualate-induced currents

ACh (50–1000 μ M) and quisqualate (Quis; 0.5–20 μ M) elicited inward currents at negative holding potentials. While Quis-induced current was stable with subsequent applications throughout the recording, the ACh-induced response sometimes declined after the first application (Figure 5b). The voltage-dependence of the ACh-induced current and the effect of antagonists were examined in cells displaying a reliably stable response to ACh.

Both Quis- and ACh-induced currents showed concentration-dependence (Figure 4). As has been previously reported (Mayer & Vyklicky, 1989; Tang *et al.*, 1989), low doses of Quis (0.5–1 μ M) evoked a current which was maintained during drug application, while higher concentrations of Quis induced a fast desensitizing, transient current which was

Figure 2 Effects of kynurenic acid and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) on miniature e.p.s.cs. Miniature e.p.s.cs recorded in picrotoxin ($20 \mu\text{M}$), tetrodotoxin ($0.3 \mu\text{M}$) and Cd^{2+} ($20 \mu\text{M}$) of which examples are shown in b(ii) (control) were strongly reduced by 0.1 mM kynurenic acid (Kyn) or $1 \mu\text{M}$ CNQX and nearly blocked by 1 mM Kyn or $10 \mu\text{M}$ CNQX.

The effects of the drugs are shown by histograms (a, b(i)) for miniature e.p.s.cs recorded in a single cell before, during and after drug application. Only cells with full recovery were considered. Sampling rate was 10 s at a frequency of 2 kHz . Analysis was done in 5 pA bins, minimum being twice the noise level. In (a) column 1 (hatched) control; column 2 (solid) 0.1 mM Kyn; column 3 (open) 1 mM Kyn. In (b(i)), column 1 (hatched) control; column 2 (solid) $1 \mu\text{M}$ CNQX; column 3 (open) $10 \mu\text{M}$ CNQX.

Figure 3 Effects of acetylcholine (ACh, $100 \mu\text{M}$) on synaptic activity. (a) In picrotoxin, ACh ($100 \mu\text{M}$) induced a barrage of e.p.s.cs without depolarizing the cell (i), while quisqualate (Quis $10 \mu\text{M}$) depolarized the same cell (ii). (b) Effects of ACh ($100 \mu\text{M}$) on e.p.s.cs recorded in the presence of picrotoxin in three different cells: (i) following the ACh-induced inward current, a barrage of e.p.s.cs is elicited; (ii) in the absence of a significant inward current, ongoing synaptic activity is reduced by ACh; (iii) in tetrodotoxin and picrotoxin, miniature e.p.s.cs are blocked after the ACh-induced inward current. (c) During and after the ACh-induced maintained inward current synaptic activity was reduced (i); this effect of ACh was antagonized by atropine (Atr) (ii).

Figure 4 (a) Acetylcholine (ACh) in $100\ \mu\text{M}$ concentration evoked a transient current while, at higher concentrations, the transient current was followed, in the same cell, by a maintained component. (c) A low concentration of quisqualate (Quis) induced only a maintained current while, in the same cell, a higher concentration induced both a transient and a maintained current component. (b,d) Concentration-effect curves for ACh- and Quis-induced currents. In (d) concentration-effect is presented for both components: (●) transient current; (○) maintained current. Concentrations given in (a,c) are in μM . Concentration-effect curves (b,d) are presented as mean (s.e. mean shown by vertical bars) from the number of cells indicated in the plot. Currents in this and all subsequent figures were recorded in the presence of tetrodotoxin.

followed by a slowly desensitizing, maintained current (Figure 4c). ACh elicited mostly a fast transient current, the rate of desensitization of which increased with increasing ACh concentration (Figure 4a). In the higher concentration range of ACh ($0.5\text{--}1.0\text{ mM}$) an additional, maintained component was sometimes apparent. In a minority of cells, ACh induced only a slow, maintained inward current.

Both transient and maintained components of ACh- and Quis-induced currents showed the same I/V characteristics (Figure 5c). The inward currents activated by ACh and Quis increased with voltage (Figure 5a,c), and currents reversed around $+10\text{ mV}$ i.e. close to the calculated equilibrium potential of $+8\text{ mV}$ for a nonselective cationic conductance (for ACh-evoked current the mean reversal determined from 10 cells was $+10.1 \pm 1.9\text{ mV}$, Quis-induced current reversed at $+8.6 \pm 1.5\text{ mV}$, $n = 6$).

ACh-evoked current could be mimicked by nicotine ($50\text{--}500\ \mu\text{M}$), the two responses showing similar I/V characteristics (Figure 6c). However, because currents induced by nicotine rapidly faded, ACh was chosen as the agonist for detailed testing. Atropine ($1\text{--}10\ \mu\text{M}$) did not diminish the current induced by ACh ($100\text{--}500\ \mu\text{M}$; $n = 6$). However, in some instances (e.g. Figure 6a,b), the current induced by ACh in the presence of atropine ($1\ \mu\text{M}$) differed with respect to the slope of its I/V curve from that without atropine suggesting interference by a muscarinic component.

Effects of antagonists on quisqualate-induced current

CNQX (1 and $5\ \mu\text{M}$), an excitatory amino acid antagonist for non-NMDA receptors, reduced maintained currents induced by low doses of α -amino-3-hydroxy-5-methylisoxazole-4-propanoic acid (AMPA, $5\text{--}20\ \mu\text{M}$, $n = 14$; Figure 7a(i)) and Quis ($0.1\text{--}1\ \mu\text{M}$, $n = 12$; Figure 7b(i)). Biphasic currents induced by $50\text{--}100\ \mu\text{M}$ AMPA ($n = 14$, Figure 7a(ii)) and by $5\text{--}20\ \mu\text{M}$ Quis

Figure 5 Characteristics of acetylcholine (ACh)- and quisqualate (Quis)-induced currents. (a) Transient and maintained inward currents elicited by ACh (1 mM) and recorded at the different holding potentials indicated. (b) Rapid rundown of an inward current induced by ACh ($100\ \mu\text{M}$) with repeated application, while a similar response evoked by Quis ($10\ \mu\text{M}$) remained stable in the same cell. (c) I/V plot for currents induced by ACh (●, $100\ \mu\text{M}$) and Quis (▲, $10\ \mu\text{M}$) in the same cell. ACh-evoked current amplitude normalised to that of Quis at -80 mV . Solid line is the regression line connecting Quis-induced currents; broken line is that connecting the ACh-induced currents.

Figure 6 Acetylcholine (ACh)-induced nicotinic receptor-mediated current (a,b). In the presence of atropine ($1\ \mu\text{M}$) the ACh ($200\ \mu\text{M}$)-induced current (○) reversed linearly, but not the current induced by ACh alone (●) in the same cell. (c) Voltage-dependence of the nicotine ($100\ \mu\text{M}$, □)- and ACh plus atropine (○)-evoked peak currents are similar when recorded in the same cell.

Figure 7 Effects of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) on α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and quisqualate (Quis) induced currents. (a(i)) CNQX (5 μ M) reduced the amplitude of the maintained current induced by 5 μ M AMPA. (a(ii)) CNQX (5 μ M) rather unexpectedly enhanced the amplitude of the maintained current which followed the transient current induced by 100 μ M AMPA. (b(i)(ii)) The same effects of CNQX were observed if 0.5 or 5 μ M Quis were applied. (c) Dose-response curve for inhibition by CNQX of the transient (●) and maintained (○) currents elicited by 20 μ M Quis. Each cell served as its own control, control set to 100%. Only cells in which current amplitude elicited by Quis before and after CNQX application were of the same size were considered. Number of cells indicated, bars represent s.e.mean.

($n = 12$, Figure 7b(ii), c) were not reduced by CNQX (1–5 μ M). On the contrary, CNQX increased the amplitude of the maintained component induced by 50–100 μ M AMPA and 5–20 μ M Quis (Figure 7a(ii), b(ii)). After increasing the CNQX concentration (20 μ M) both current components were reduced (Figure 7c). APV (20 μ M, $n = 3$) had no effect on the transient or on the plateau current. Kynurenic acid (0.1–1 mM, $n = 12$) also reduced maintained currents induced by low doses of quisqualate (Figure 8a(i)). However, only the transient component of the biphasic current induced by 20 μ M Quis was diminished, while maintained currents were not affected (Figure 8a(ii)). ACh-(100 μ M) induced current was not affected by CNQX (20 μ M; $n = 8$ and 100 μ M; $n = 3$) or by kynurenic acid (1 mM; $n = 4$).

Effects of antagonists on nicotine-induced current

Mecamylamine, a ganglionic nicotinic antagonist, suppressed ACh-induced currents in a dose-dependent manner. The effect was reversible and did not show voltage-dependence (Figure 9). The same effect of mecamylamine (100 μ M) was observed when tested against various ACh concentrations (200–500 μ M, $n = 11$). Currents induced by 0.5 μ M Quis were unaffected by mecamylamine (100 μ M; $n = 6$).

Figure 8 Effect of kynurenic acid (Kyn) on quisqualate (Quis) induced currents. (a(i)) Kyn (1 mM) reduced the amplitude of the maintained current induced by 0.5 μ M Quis. (a(ii)) Kyn (0.5 mM) reduced the transient component of the current induced by Quis (5 μ M) leaving the maintained component unaffected. (b) Dose-response curve for inhibition by Kyn of the transient (●) and maintained (○) currents elicited by 5 μ M Quis. Same protocol as in Figure 7c.

Hexamethonium (up to 400 μ M) had in most instances no effect on ACh-induced currents (50–500 μ M, $n = 12$). Only in two cells, in which the ACh-induced current was of the slow-maintained type, was a voltage-dependent blockade by hexa-

Figure 9 Current induced by acetylcholine (ACh) is reversibly and dose-dependently reduced by mecamylamine (inset for 100 μ M ACh and 10 μ M mecamylamine (Mec)). Each point is the mean from 4 cells; bars show s.e.mean.

Figure 10 Voltage-dependent reduction by hexamethonium of acetylcholine (ACh)- and quisqualate Quis-induced currents. (a) I/V plot for currents induced by ACh (100 μ M) (□) and ACh (100 μ M) simultaneously applied with hexamethonium (50 μ M) (■). (b) I/V plot for Qui (0.5 μ M)-induced current (□) and the current induced by simultaneous application of Quis (0.5 μ M) and hexamethonium (400 μ M) (■). In (a) and (b) actual recordings of the cells plotted are shown to the right. Membrane potential indicated at the beginning of each trace.

methonium (50 μ M) observed (Figure 10a). Hexamethonium also attenuated maintained currents evoked by a low concentration of Quis (0.5 μ M). This effect was concentration-dependent; 50 μ M hexamethonium diminished the Quis-current by $23 \pm 5.6\%$ ($n = 4$) and 400 μ M decreased the current by $65 \pm 10\%$ ($n = 4$) at a holding potential of -80 mV. Hexamethonium-induced blockade of the Quis current was voltage-dependent ($n = 3$, Figure 10b). Currents resulting from application of higher Quis concentrations, consisting of a transient and a maintained component, were not affected by hexamethonium ($n = 4$).

Discussion

The non-NMDA receptor antagonists CNQX and kynurenic acid, but not the NMDA antagonist APV and the nicotinic antagonists mecamylamine and hexamethonium, reduce network generated e.p.s.cs and miniature e.p.s.cs in the mid-brain culture, suggesting that e.p.s.cs are mediated by an EAA. Despite the apparent ability of the antagonists to discriminate non-NMDA receptor-mediated e.p.s.cs from nicotinic receptor-mediated e.p.s.cs, their interaction with their respective agonist is rather complicated and unforeseen.

Identification of receptors involved in the generation of e.p.s.ps in mammalian CNS is based primarily on their blockade by antagonists such as CNQX and kynurenic acid and the ineffectiveness of nicotinic receptor antagonists. This approach requires selectivity and specificity of the antagonists. Strictly speaking, suitability of an antagonist should be evaluated while testing its effect on the signals induced by various synaptically released transmitters. Since in midbrain culture there was no evidence of nicotinic receptor-mediated spontaneous e.p.s.cs, this approach was not available. Instead, we tested the effects of antagonists on responses produced by the exogenously applied agonists, Quis and ACh. By comparison with AMPA-induced currents, it was ensured that Quis effects under study were mediated by the so-called AMPA-receptor type.

Both e.p.s.cs and Quis and nicotinic receptor-induced currents reversed at the same membrane potential. Currents evoked by Quis and ACh contained a desensitizing component, which is considered characteristic of ACh and EAA receptors mediating synaptic responses (Katz & Thesleff, 1957; Trussell *et al.*, 1988; Mayer & Vyklicky, 1989; Tang *et al.*, 1989). However, Quis- and ACh-induced currents differed in that ACh induced much smaller currents than Quis. Not all cells responding to Quis and exhibiting e.p.s.cs responded to ACh.

The difficulty in comparing synaptically and exogenously induced currents becomes apparent when considering the transient and maintained components of the Quis-induced current. The transient component, induced by activation of a high-conductance channel-coupled receptor, is elicited by Quis concentrations above 1 μ M (Tang *et al.*, 1989). However, CNQX and kynurenic acid reduced the transient current only at concentrations much higher than those required to reduce spontaneous e.p.s.cs. Since both CNQX and kynurenic acid are considered competitive antagonists (Blake *et al.*, 1988; 1989; Bertolino *et al.*, 1989) this difference may indicate a lower concentration, a lower affinity or a faster removal of the synaptically released ligand compared with exogenous ligands. The comparison of antagonists' actions on synaptic and evoked responses is further complicated by their differential effects on transient and maintained components of the evoked current. CNQX eventually enhanced the maintained current without affecting the transient current, while kynurenic acid reduced the transient but not the maintained component. It has been suggested that the ratio between transient and maintained components of Quis-current is governed by desensitization, which increases with an increase in Quis or AMPA concentration (Mayer & Vyklicky, 1989). Because desensitization of glutamate receptors may be fast (Franke *et al.*, 1987) and receptors for glutamate are primarily located on dendrites (Swandulla & Misgeld, 1990), in whole cell recording some of the current may be entirely lost by desensitization. Therefore, effects of CNQX may reflect in part removal of desensitization. Such an action may be in line with a competitive antagonism. By the same token, kynurenic acid, which did not increase the maintained current, may not be a purely competitive antagonist.

Since nicotinic receptor-mediated responses did not have their synaptic counterpart in our experimental model, characterization of the nicotinic antagonists could not be complete, and the possibility of the blockade of nicotinic e.p.s.ps by CNQX and kynurenic acid cannot be excluded. However, our study showed that high concentrations of non-NMDA antagonists did not affect the small current induced by low concentrations of ACh. Likewise, high concentrations of the nicotinic antagonist mecamylamine did not affect the response to low concentrations of Quis and had no effect on e.p.s.cs.

The effect of hexamethonium raises another issue. Hexamethonium did not reduce transient nicotinic and Quis currents, but reduced in a voltage-dependent manner maintained responses to both agonists. Hexamethonium is known as a partial channel blocking agent (Ascher *et al.*, 1979). Therefore, its ability to reduce Quis- and nicotine-induced currents may

be due to a similarity of Quis- and nicotinic-ionic channels. Despite this apparent lack of selectivity, hexamethonium is a ganglionic blocking agent which does not seem to reduce EAA-mediated e.p.s.ps in central neurones.

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