Bicuculline blocks nicotinic acetylcholine response in isolated intermediate lobe cells of the pig

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1 The effect of bicuculline on nicotinic acetylcholine (ACh) responses in isolated intermediate lobe (IL) cells of the pig was investigated by use of patch-clamp techniques. Bicuculline was found to reduce ACh-evoked whole-cell currents (I_{ACh}) in all cells tested ($n = 40$).

2 The blocking effect of bicuculline on I_{ACh} was dose-dependent, the concentration producing half-maximal blockade being $43.8 \mu\text{M}$.

3 The blockade of I_{ACh} by bicuculline was not voltage-dependent at membrane potentials above -60 mV , but a slight voltage-dependence was observed at holding potentials (HP) of -80 and -100 mV .

4 The inhibitory effect of bicuculline on I_{ACh} was partially competitive at a HP of -60 mV .

5 Neither SR 95531, a pyridazinyl γ -aminobutyric acid derivative, nor *t*-butylbicyclopophosphorothionate (TBPS) blocked I_{ACh} in IL cells.

6 It is concluded that bicuculline interacts directly with the ACh receptor-ionophore complex on porcine IL cells.

Introduction

The selective antagonism of bicuculline to the γ -aminobutyric acid (GABA)-mediated responses in vertebrate systems has been studied, *in vivo* and *in vitro*, by the measurement of several parameters such as cell firing, GABA-gated Cl^- currents and specific binding of GABA_A ligands (Reviews by Nistri & Constanti, 1979; Bormann, 1988). Stable radiolabelled derivatives of bicuculline have also been used to assay biochemically the kinetics of GABA receptors (Olsen & Snowman, 1983). Thus, bicuculline is applied as an important tool in tracking the physiologically relevant function of GABA-ergic synaptic activities in various experimental situations ranging from electrophysiological studies of post and pre-synaptic inhibition in neural networks, to integrated studies of behavioural regulation (for review see, Krosgaard-Larsen *et al.*, 1988).

Using the pars intermedia of the pituitary as a model to study neural control of endocrine systems, we and others have demonstrated the functional role of GABA postsynaptic actions in the IL cells, and, the presence on these cells of GABA_A and GABA_B receptors with different sensitivity to bicuculline (Taraskevich & Douglas, 1982; Demeneix *et al.*, 1984; 1986a). *In vitro* electrophysiological studies (Davis *et al.*, 1985; Williams *et al.*, 1989) showed that stimulation of pituitary stalk produced in the pars intermedia, a fast depolarization (excitatory postsynaptic potential e.p.s.p.), followed by a slow hyperpolarization (inhibitory postsynaptic potential, i.p.s.p.). The latter was concluded to be a dopaminergic inhibitory effect. The fast e.p.s.p. was interpreted as mediated by GABA_A receptors, as it was suppressed by bicuculline. We raised another possibility in our recent study, where we reported a neuronal-type nicotinic acetylcholine (ACh) receptor in the IL cells maintained in primary culture (Zhang & Feltz, 1990). ACh, as well as nicotine induced depolarization and action potentials in the cells by opening a cation selective conductance. This effect was sensitive to (+)-tubocurarine, hexamethonium and mecamylamine, but not to α -bungarotoxin (Zhang & Feltz, 1990).

In the present study, we investigated the effect of the GABA_A antagonist, bicuculline on these nicotinic ACh receptors in isolated lobe cells of the pig.

Methods

Preparation

The primary culture of porcine IL cells was prepared as described previously (Demeneix *et al.*, 1986b). In brief, porcine pituitary glands were collected from the local abattoir, the anterior lobes were removed by careful dissection and the IL cells were dissociated with collagenase (1 mg ml^{-1}) and trypsin (0.5 mg ml^{-1}). The cell suspension was purified on a bovine serum albumin gradient. Cells were collected and resuspended in culture medium containing 65% Dulbecco's modified Eagle's medium (DMEM), 25% HAM's nutrient mixture F12 and 10% foetal calf serum. The suspension was plated in Costar 35 mm dishes and was kept in a humidified atmosphere of 95% air and 5% CO_2 at 37°C for up to 10 days.

Experiments were carried out between 2 to 8 days after plating. Only isolated cells were chosen for recording.

Recording and analysis

Experiments were performed at room temperature ($15\text{--}18^\circ\text{C}$). Cells were recorded in the whole-cell configuration (Hamill *et al.*, 1981) with a List patch-clamp amplifier (EPC-7). Recording pipettes were pulled from soft capillary glass (WV-Mainz) and coated with Sylgard (Rhodorsil RTV-141, Rhone Poulenc). When filled with recording solution, electrodes had a resistance of 3 to $5 \text{ M}\Omega$. Current and voltage signals were monitored on a digitizing oscilloscope (Tektronix, 5223) and stored simultaneously on videotape (Sony, PCM-701: bandwidth: d.c. to 10 kHz). Data were usually filtered at 250 Hz (8-pole Bessel-type: -3 dB), and digitized at 500 Hz with a data acquisition board (scientific solutions, 40 kHz) operated by an IBM compatible computer (Olivetti M28).

Solutions

The extracellular solution contained (in mM): NaCl 127, KCl 3, MgCl_2 2, CaCl_2 2, HEPES/NaOH 5 (pH 7.4). The intracellular solution contained (in mM): CsCl 130, MgCl_2 2, CaCl_2 0.9, EGTA 10, HEPES/CsOH 5 (pH 7.2). Acetylcholine chloride and bicuculline methiodide were obtained from Sigma. *t*-Butylbicyclopophosphorothionate (TBPS) was obtained from New England Nuclear Corp. The synthetic antagonist, 2-(carboxy - 3' - propyl) - 3 - amino - 6 - para - methoxyphenyl -

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Figure 1 The effect of bicuculline methiodide on acetylcholine (ACh)-induced currents in an isolated IL cell voltage-clamped at -60 mV. Control response to ACh $100 \mu\text{M}$ ($\square$), response to ACh $100 \mu\text{M}$ in the presence of bicuculline methiodide $20 \mu\text{M}$ ($\circ$) and recovery (Δ). Recordings were filtered at 100 Hz and digitized at 200 Hz.

pyridazinium bromide (SR 95531) was generously provided by SANOFI Recherche (Montpellier). Drugs were applied with a microperfusion system (Fenwick *et al.*, 1982).

Results

Experiments were carried out on 40 cells from 9 cell cultures. The blocking effect of bicuculline was observed in all cells tested. Figure 1 illustrates an example of the effect of bicuculline on nicotinic ACh responses in IL cells. Bicuculline reversibly reduced ACh-induced peak current by 39% on the cell voltage-clamped at -60 mV.

The dose-dependence of the effect of bicuculline was examined on 5 cells. Figure 2 shows the dose-responses of bicuculline for the inhibition of ACh-evoked currents. The 50% inhibition (K_D) was observed at $43.8 \mu\text{M}$.

Figure 2 The dose-dependence of the effect of bicuculline methiodide on the peak current evoked by acetylcholine $100 \mu\text{M}$. Data obtained from 5 cells held at -60 mV. Vertical bars indicate the s.e.mean.

Figure 3 Current-voltage relation of responses induced by acetylcholine $100 \mu\text{M}$ in the absence ($\bullet$) and presence ($\square$) of bicuculline methiodide $20 \mu\text{M}$. Results obtained from 4 cells; vertical bars show s.e.mean.

Figure 3 shows the voltage-dependence of the effect of bicuculline. Cells were held at different membrane potentials. Bicuculline methiodide was applied simultaneously with ACh by microperfusion. The action of bicuculline was not voltage-sensitive at membrane potentials above -60 mV. However, from -80 to -100 mV, a slight voltage-dependence was observed, the blocking effect being more important at these holding potentials.

Dose-response curves to ACh were established on cells voltage-clamped at -60 mV. The dose-response curve in the absence of bicuculline (Figure 4) was sigmoid with a half maximal value (EC_{50}) of $112 \mu\text{M}$. Bicuculline ($10 \mu\text{M}$, Figure 4) shifted the curve to the right by increasing the EC_{50} to $352 \mu\text{M}$. The maximal response was slightly decreased by 8% in the presence of bicuculline ($10 \mu\text{M}$).

Figure 4 Acetylcholine (ACh) dose-response curves. Control ($\bullet$); with bicuculline methiodide $10 \mu\text{M}$ ($\blacksquare$). Cells were held at -60 mV. Data were mean values obtained in 4 experiments; vertical bars show s.e.mean.

Figure 5 The effect of SR 95531 on responses to stimulation of GABA receptors (a) and acetylcholine receptors (b) (chart display). Results obtained from the same cell with a holding potential of -60 mV .

It is important to know whether other GABA antagonists are able to block the responses of ACh as well as bicuculline. SR 95531 has been demonstrated to be a potent competitive antagonist of GABA_A receptors (Wermuth & Biziere, 1986; Mienville & Vicini, 1987; Hamann *et al.*, 1988). Figure 5a illustrates its efficiency on GABA responses in IL cells. However, SR 95531 failed to reduce ACh-induced responses (Figure 5b and in 7 other cells). TBPS, a non-competitive antagonist of GABA_A receptors (Squires *et al.*, 1983), was not able to block the cholinergic responses (3 cells).

Discussion

Following its description as an antagonist of GABA in vertebrates, bicuculline has been widely studied in a variety of invertebrate and vertebrate preparations in the context of its effect on GABA_A responses. The studies in invertebrates have given contradictory results (for review see, Nistri & Constanti, 1979). The effect of bicuculline appeared to be species and tissue-dependent and was much less potent than in vertebrate systems. At high concentrations ($>10^{-4}\text{ M}$) bicuculline was shown to antagonize GABA responses in invertebrate muscles (Takeuchi & Onodera, 1972; Earl & Large, 1974; Shank *et al.*, 1974). However, binding studies failed to demonstrate it as a competitive antagonist of GABA (Olsen *et al.*, 1976; Mann & Enna, 1980). Furthermore, with voltage-clamp techniques, Benson (1988) showed that bicuculline was inactive in blocking GABA-induced Cl^- conductance on locust ganglion neurones, whereas it did inhibit ACh-evoked cation channels in these cells. This is, to our knowledge, the only example of blockade of a cation conducting channel by bicuculline, via a competition with a ligand other than GABA.

Despite its role as a potent competitive antagonist of the GABA_A receptor in the mammalian nervous system, a number

of studies have questioned the selectivity of bicuculline for GABA (Godfraind *et al.*, 1970; Straughan *et al.*, 1971). Experiments *in vivo* have shown bicuculline to be a weak antagonist of GABA-ergic synaptic inhibition on rat cortical neurones (Straughan *et al.*, 1971; Hill *et al.*, 1973). These investigations prompted biochemical studies *in vitro* revealing bicuculline to be a competitive inhibitor of acetylcholinesterase (AChE) in mammalian brain (Svenneby & Roberts, 1973; Breuker & Johnston, 1975; Olsen *et al.*, 1976), results which were confirmed by electrophysiological recordings in rat central neurones (Miller & McLennan, 1974). However, neuronal-type nicotinic receptors in the central nervous system (CNS) were neglected in these early studies, and the effect of bicuculline was attributed to an indirect potentiation of muscarinic response via the inhibition of AChE. Our studies in IL cells demonstrate a direct interaction of bicuculline with the ACh receptor-channel complex on vertebrate cells. This finding corroborates the previous findings and suggests a possible blocking effect of bicuculline on ACh receptors, in particular the neuronal-type ACh receptors in mammalian nervous systems. Furthermore, our results emphasize the case for using synthetic GABA antagonists, such as the pyridazinyl GABA derivatives, in the physiological studies of synaptic transmissions in mammalian CNS.

In locust ganglion neurones, the blocking effect of bicuculline was independent of membrane potential (Benson, 1988). The I-V curves obtained from porcine IL cells showed a different profile. A slight voltage-dependence could be observed at holding potentials over the range of -60 to -100 mV . This suggests a possible channel blocking effect of bicuculline.

The conventional dose-response data of our experiments indicate that at -60 mV , the effect of bicuculline ($10\text{ }\mu\text{M}$) on the I_{ACh} is at least partially competitive. The dose-response curve was shifted to the right in the presence of bicuculline.

The slight decrease of maximal response might be due to a weak non-competitive channel blocking effect of bicuculline.

It is of interest to mention that several other AChE inhibitors (e.g. neostigmine and physostigmine) can also antagonize nicotinic ACh responses (Slater *et al.*, 1986; Sadoshima *et al.*, 1988). This effect is supposed to be related to their structural similarity with ACh. ACh, bicuculline methiodide and other quaternary derivatives of bicuculline bear some similarity in structure, including the positive charged quaternary ammonium group. This analogy could be the basis of the interaction of bicuculline with ACh receptors.

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