Effects of potassium channel openers and their antagonists on rat locus coeruleus neurones

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1 Intracellular recordings were obtained from a pontine slice preparation of the rat brain containing the locus coeruleus (LC). Two openers of ATP-sensitive potassium (K_{ATP}) channels, RO 31-6930 (10 μ M) and cromakalim (100 μ M) decreased the spontaneous discharge of action potentials without altering their amplitude or duration. Neither compound changed the resting membrane potential.

2 Of two K_{ATP} channel blockers, tolbutamide (300 μ M) increased the firing rate, while glibenclamide (3 μ M) only tended to do so. In addition, both compounds antagonized the effect of RO 31-6930 (10 μ M). Neither glibenclamide (3 μ M) nor tolbutamide (300 μ M) altered the resting membrane potential.

3 Tetrodotoxin (0.5 μ M) depressed the firing, but did not influence the inhibitory action of RO 31-6930 (10 μ M). The excitatory amino acid antagonist, kynurenic acid (500 μ M), did not change the spontaneous discharge of action potentials.

4 Small shifts (2–4 mV) of the membrane potential by hyper- or depolarizing current injections markedly decreased and increased the firing rate, respectively.

5 Noradrenaline (100 μ M) hyperpolarized the cells and decreased their input resistance. This effect was not antagonized by glibenclamide (3 μ M) or tolbutamide (300 μ M). Ba^{2+} (2 mM), a blocker of both ATP-sensitive and inwardly rectifying potassium channels, abolished the effects of RO 31-6930 (10 μ M) and noradrenaline (100 μ M).

6 These data suggest that K_{ATP} channels are present on the noradrenergic LC neurones, but are not coupled to α_2 -adrenoceptors.

Keywords: ATP-sensitive potassium channel; cromakalim; RO 31-6930; locus coeruleus; glibenclamide; tolbutamide

Introduction

Recently a novel type of K^+ channel has been described, which is blocked by intracellular ATP (Noma, 1983; Ashcroft, 1988; Dunne & Petersen, 1991). This ATP-sensitive K^+ conductance ($I_{K(ATP)}$) is present not only in pancreatic β -cells, heart, skeletal and smooth muscle, but also in various neurones (Ashcroft & Ashcroft, 1990; de Weille & Lazdunski, 1990). A certain class of drugs, the potassium channel openers (e.g. cromakalim and RO 31-6930), activate $I_{K(ATP)}$, while sulphonylurea antidiabetics (e.g. glibenclamide and tolbutamide) inhibit it, thereby antagonizing the effects of the channel openers (Cook, 1988).

[³H]-glibenclamide has been shown to bind with high selectivity to K_{ATP} channels of central neurones (Bernardi *et al.*, 1988). Although these binding sites are widely distributed throughout the brain (Mourre *et al.*, 1989; Gehlert *et al.*, 1991), the highest densities have been observed in the substantia nigra (SN) (Mourre *et al.*, 1989; Treherne & Ashford, 1991). Most neurones of the SN zona compacta fire spontaneous action potentials at a constant rate followed by prominent afterhyperpolarizations (Kita *et al.*, 1986; Silva & Bunney, 1988; Lacey *et al.*, 1988). SN cells possess dopamine D_2 -autoreceptors, which are coupled via a guanosine 5'-triphosphate- (GTP) binding protein to inwardly rectifying potassium channels (Lacey *et al.*, 1988). Dopamine or synthetic D_2 -agonists such as quinpirole cause hyperpolarization, and inhibition of the spontaneous firing (Lacey *et al.*, 1988). In acutely dissociated cells, the quinpirole-induced conductance is reversed by tolbutamide suggesting the involvement of $I_{K(ATP)}$ (Roeper *et al.*, 1990). A decrease in intracellular ATP or the extracellular application of cromakalim also hyperpolarizes SN neurones confirming the presence of K_{ATP} channels (Häusser *et al.*, 1991). By contrast, in brain slices containing the SN, glibenclamide does not antagonize the

effect of dopamine, and cromakalim is without effect (Hicks & Henderson, 1992).

Noradrenergic neurones of the nucleus locus coeruleus (LC) exhibit electrophysiological characteristics similar to dopaminergic SN cells. LC neurones discharge spontaneous action potentials with a pacemaker-like regularity; the stimulation of somatic α_2 -adrenoceptors inhibits the firing (Cedarbaum & Aghajanian, 1977; Henderson *et al.*, 1982). These receptors hyperpolarize the membrane by increasing an inwardly rectifying potassium conductance (Aghajanian & VanderMaelen, 1982; Williams *et al.*, 1985; 1988).

Because of the similarities between SN and LC neurones, we searched for the presence of an ATP-sensitive potassium conductance in the LC. Moreover, we investigated whether the α_2 -autoreceptors of LC cells are coupled to K_{ATP} channels.

Methods

Brain slice preparation

Midpontine slices of the rat brain were prepared and maintained as previously described (Illes & Nörenberg, 1990; Regenold & Illes, 1990). In brief, male Wistar rats (150–200 g) were anaesthetized with ether and decapitated. Coronal slices of about 400 μ m thickness, containing the caudal part of the LC were prepared in oxygenated medium at 1–4°C with a Lancer vibratome. A single slice was placed in a recording chamber and was superfused at a rate of 2 ml min⁻¹ with medium saturated with 95% O₂ plus 5% CO₂ and maintained at 35–36°C. The medium had the following composition (in mM): NaCl 126, KCl 2.5, NaH₂PO₄ 1.2, MgCl₂ 1.3, CaCl₂ 2.4, NaHCO₃ 25 and glucose 11. Ascorbic acid (0.3 mM) and EDTA (0.03 mM) were also present in order to prevent oxidation of noradrenaline. When

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MgCl₂ was added, NaCl was reduced to maintain a similar osmolality. No osmotic compensation was made for BaCl₂.

Identification of LC neurones and intracellular recording

LC neurones were identified under a binocular microscope at the ventrolateral border of the fourth ventricle. LC cells were distinguished from neighbouring mesencephalic trigeminal neurones by their electrophysiological properties including spontaneous firing at a frequency of 0.2–5 Hz and a hyperpolarizing response to noradrenaline (Henderson *et al.*, 1982; Williams *et al.*, 1985).

Recording of the membrane potential and current injection was carried out with glass micro-electrodes filled with KCl (2 M) (tip resistance, 70–120 MΩ) using a high impedance pre-amplifier and a bridge-circuit (Axoclamp 2A). In some experiments hyper- or depolarizing currents were passed through the micro-electrode in order to induce small (2–4 mV) shifts of the membrane potential. Current injection was for 2 min at 5–8 min intervals. The changes in membrane potential were evoked sequentially (–2, –4, 2 and 4 mV) in order to determine the corresponding alterations of the firing rate. Another group of LC cells was constantly hyperpolarized by about 20 mV. In addition, hyperpolarizing current pulses of constant amplitude and 250 ms duration were delivered at a frequency of 0.5 Hz. The apparent input resistance was calculated from the peak potential change produced. The membrane potential was determined on withdrawal of the micro-electrode from the cell at the end of each experiment. Changes in the membrane potential were displayed on a Gould RS 3200 pen-recorder and in addition stored on tape (Racal Store 4). The action potentials were plotted by means of a Gould 1425 digital oscilloscope on a Gould Colorwriter 6120.

Application of drugs

Drugs were applied by changing the superfusion medium by means of three-way taps. At the constant flow rate of 2 ml min^{–1} about 30 s were required until the drugs reached the bath. In some experiments we used only K_{ATP} channel openers (cromakalim, RO 31-6930) or blockers (glibenclamide, tolbutamide); in this case the superfusion times were 15 and 25 min, respectively. When the interaction of these

compounds was studied, we added the blockers for 10 min at first, and then added the openers for another 15 min in the continuous presence of the blockers. A similar time-schedule was adopted when the influence of tetrodotoxin or Ba²⁺ was tested on the effect of RO 31-6930. Kynurenic acid was superfused for 10 min.

Before starting an experiment, the LC neurones were identified by their hyperpolarizing response to noradrenaline. When noradrenaline was applied both before and 25 min after the addition of glibenclamide or tolbutamide, the α-adrenoceptor agonist was present in the superfusion medium for 2 min each time. In some cases the effect of noradrenaline was determined on the input resistance both before and 10 min after the addition of glibenclamide.

Materials

The following drugs were used: RO 31-6930 (2-(6-cyano-2,2-dimethyl-2H-1-benzopyran-4-yl)pyridine-1-oxide) (M. Eltze, Byk Gulden, Konstanz, Germany); glibenclamide, tolbutamide (H. Englert; Hoechst, Frankfurt am Main, Germany); kynurenic acid (RBI, Natick, MA, U.S.A.); (–)-noradrenaline hydrochloride, tetrodotoxin (Sigma, Deisenhofen, Germany); cromakalim (S. Pathak; Smith, Kline & Beecham, Betchworth, Surrey).

Stock solutions (1–100 mM) of all compounds were prepared in the following solvents: distilled water (noradrenaline, tetrodotoxin), 70% ethanol (RO 31-6930), 0.2 N NaOH (glibenclamide, tolbutamide) or medium (cromakalim, kynurenic acid). Further dilutions were made with medium. Equivalent quantities of the solvents had no effect.

Statistics

Means ± s.e.mean are given throughout. The Kruskal-Wallis analysis followed by the Mann-Whitney-test was used for the comparison of the means and the comparison of the means with zero. A probability level of 0.05 or less was considered to be statistically significant. In the case of multiple comparisons, the significance level was adjusted according to the method of Bonferroni (Wallenstein *et al.*, 1980).

Table 1 Effects of K_{ATP} channel openers and blockers on the frequency of spontaneous action potentials in rat locus coeruleus neurones and the interaction of these compounds with each other

Treatment	μM	n	Change in frequency (%)		
			0–10 min	0–15 min	10–25 min
None		9	11.6 ± 10.2	15.0 ± 16.0	–0.2 ± 10.2
RO 31-6930	10	12		–72.1 ± 8.5 ^a	
Cromakalim	100	6		–71.2 ± 12.3 ^a	
Glibenclamide	3	5	70.2 ± 32.1		8.6 ± 11.2
Glibenclamide	3	11	90.4 ± 40.3		
+ RO 31-6930	10	11			1.7 ± 11.2 ^b
Tolbutamide	300	5	137.8 ± 48.5 ^a		–1.8 ± 6.4
Tolbutamide	300	6	161.7 ± 74.4 ^a		
+ RO 31-6930	10	6			12.8 ± 9.3 ^b
Tetrodotoxin	0.5	5	–52.1 ± 16.2 ^a		
+ RO 31-6930	10				–59.6 ± 13.3 ^a
Ba ²⁺	2000	6	–18.8 ± 8.0		
+ RO 31-6930	10	6			7.4 ± 13.8 ^b

No effect of tetrodotoxin on the RO 31-6930-induced decrease in firing rate was observed.

In some experiments only K_{ATP} channel openers (cromakalim, RO 31-6930) or blockers (glibenclamide, tolbutamide) were used; in this case the superfusion times were 15 and 25 min, respectively. When the interaction of these compounds was studied, at first the blockers were added for 10 min, and then the openers for another 15 min in the continuous presence of the blockers. A similar time-schedule was adopted when the influence of tetrodotoxin or Ba²⁺ was tested on the effect of RO 31-6930. The percentage changes in frequency were calculated with respect to 0 or 10 min, as indicated. Means ± s.e.mean of *n* experiments are shown.

^a*P* < 0.05–0.01: significant differences from corresponding changes in the absence of drugs; ^b*P* < 0.01: significant differences from the effect of RO 31-6930 alone.

Figure 1 Effect of RO 31-6930 on the membrane potential and firing rate of rat locus coeruleus neurones and interaction with glibenclamide. The full height of action potentials was not reproduced by the pen recorder. The inhibitory effect of RO 31-6930 (RO; $10\ \mu\text{M}$) on the spontaneous firing was reversed by glibenclamide (Gli; $3\ \mu\text{M}$). The superfusion times of both compounds are indicated by the horizontal bars. The intervals between the traces are shown.

Results

A total of 84 LC cells included in this study had a resting membrane potential of $-56.2 \pm 0.6\ \text{mV}$ and a spontaneous firing rate of $1.5 \pm 0.1\ \text{Hz}$. In 9 experiments, measurements were carried out for 25 min in the absence of any drug; under these conditions both the membrane potential ($2.1 \pm 0.9\ \text{mV}$ depolarization; $P > 0.05$) and the discharge of action potentials (Table 1) were stable.

Effects of K_{ATP} channel openers

In a first series of experiments, RO 31-6930 ($10\ \mu\text{M}$) depressed the firing rate 15 min after its application by about 70% (Figure 1, Table 1). By contrast, the membrane potential of the LC cells was not altered by RO 31-6930 ($10\ \mu\text{M}$) ($0.2 \pm 1.2\ \text{mV}$ hyperpolarization; $n = 12$; $P > 0.05$) when compared with the corresponding change measured under drug-free conditions ($1.0 \pm 0.4\ \text{mV}$ depolarization; $n = 9$). Moreover, the amplitude and duration of action potentials, as well as the afterhyperpolarization following a spike were stable in spite of the presence of RO 31-6930 ($10\ \mu\text{M}$) (Figure 2; $n = 10$). Cromakalim ($100\ \mu\text{M}$) decreased the firing rate to a similar extent as RO 31-6930 ($10\ \mu\text{M}$) (Table 1), and failed to alter the membrane potential ($0.5 \pm 2.0\ \text{mV}$ depolarization; $n = 6$; $P > 0.05$). Neither the amplitude and duration of action potentials, nor the afterhyperpolarization were changed by cromakalim ($100\ \mu\text{M}$) ($n = 4$).

Effects of K_{ATP} channel blockers and their interaction with K_{ATP} channel openers

Glibenclamide ($3\ \mu\text{M}$) did not change the frequency of action potentials in some cells (Figure 3), but increased it in others.

In a total of 16 neurones, glibenclamide ($3\ \mu\text{M}$) tended to increase the firing rate, although this effect did not reach statistical significance ($84.1 \pm 29.0\%$; $n = 16$; $P > 0.05$) when compared with drug-free controls ($11.6 \pm 10.2\%$; $n = 9$; see also Table 1). We noticed already in a few ($n = 3$) preliminary experiments that the inhibitory effect of RO 31-6930 ($10\ \mu\text{M}$) could be counteracted by subsequently applied glibenclamide ($3\ \mu\text{M}$) (Figure 1). The period of very fast spiking soon after the addition of glibenclamide ($3\ \mu\text{M}$) was probably an instability of neuronal activity, since it was observed only in one cell. In most cases we used the reversed protocol; a 10 min contact with glibenclamide ($3\ \mu\text{M}$) was followed by a 15 min co-application of RO 31-6930 ($10\ \mu\text{M}$) and glibenclamide ($3\ \mu\text{M}$). Under these conditions the K_{ATP} channel opener failed to produce any inhibition (Figure 3, Table 1).

In contrast to glibenclamide ($3\ \mu\text{M}$), tolbutamide ($300\ \mu\text{M}$) markedly facilitated the discharge of action potentials (Figure 4, Table 1). Moreover, the inhibitory effect of RO 31-6930 ($10\ \mu\text{M}$) was abolished in the concomitant presence of tolbutamide ($300\ \mu\text{M}$) (Figure 4, Table 1).

Neither glibenclamide ($3\ \mu\text{M}$) ($0.1 \pm 0.2\ \text{mV}$ depolarization; $n = 16$; $P > 0.05$) nor tolbutamide ($300\ \mu\text{M}$) ($0.6 \pm 0.5\ \text{mV}$ hyperpolarization; $n = 11$; $P > 0.05$) influenced the membrane potential of LC cells on their own; the corresponding depolarization measured under drug-free conditions was $0.5 \pm 0.3\ \text{mV}$ ($n = 9$).

Effect of current injection

The firing rate of 5 LC neurones was $2.2 \pm 0.7\ \text{Hz}$ at the resting membrane potential of $-54.3 \pm 2.0\ \text{mV}$. Already slight (2 and 4 mV) hyperpolarizations markedly decreased the firing rate by $48.4 \pm 7.7\%$ and $83.1 \pm 8.5\%$, respectively.

Figure 2 Effect of RO 31-6930 on spontaneous action potentials of locus coeruleus neurones. Each tracing shows the average of four spikes before (a) and 10 min after (b) the application of RO 31-6930 (RO; 10 μ M). Note that the time-scale of (i) is different from that of (ii).

In contrast, similar depolarizations (2 and 4 mV) increased the firing rate by $67.5 \pm 5.7\%$ and $120.5 \pm 21.3\%$, respectively. All changes were statistically significant ($P < 0.05$).

Effects of tetrodotoxin, and a low Ca^{2+} , high Mg^{2+} medium, as well as kynurenic acid

Tetrodotoxin (TTX; 0.5 μ M) decreased the amplitude and prolonged the duration of the action potentials ($n = 5$). It also markedly depressed the firing rate (Table 1). However, the inhibitory potency of RO 31-6930 (10 μ M) was unaffected in the presence of TTX (0.5 μ M). In order to block all synaptic inputs to LC neurones, several types of low Ca^{2+} (0.3 mM), high Mg^{2+} (5–20 mM) media were used. Unfortunately all treatments depolarized the cells, and after a transient increase produced a complete blockade of the spontaneous action potential discharge. Thus, under these conditions it was not possible to investigate the effects of K_{ATP} channel openers on the firing rate.

Kynurenic acid (500 μ M) did not change the frequency of spontaneous action potentials ($4.8 \pm 13.7\%$ increase; $n = 5$; $P > 0.05$) when compared with measurements in the absence of drugs ($11.6 \pm 10.2\%$ increase; $n = 9$).

Effect of noradrenaline in the presence of K_{ATP} channel blockers or Ba^{2+}

Noradrenaline (100 μ M) hyperpolarized LC neurones to the same extent both before (13.5 ± 3.7 mV) and 25 min after superfusion with glibenclamide (3 μ M) (12.4 ± 2.7 mV; $n = 5$; $P > 0.05$). Tolbutamide (300 μ M) also did not alter the effect

Figure 3 Effect of glibenclamide on the membrane potential and firing rate of locus coeruleus (LC) neurones and interaction with RO 31-6930. Glibenclamide (Gli; 3 μ M) slightly increased the spontaneous firing and antagonized the inhibitory effect of RO 31-6930 (RO; 10 μ M). The LC cell was identified by its sensitivity to noradrenaline (NA; 100 μ M). The superfusion times of all compounds are indicated by the horizontal bars. The intervals between the traces are shown. Note that recording has two different time-scales.

Figure 4 Effect of tolbutamide on the membrane potential and firing rate of locus coeruleus (LC) neurones and interaction with RO 31-6930. Tolbutamide (Tol; $300 \mu\text{M}$) increased the spontaneous firing and antagonized the inhibitory effect of RO 31-6930 (RO; $10 \mu\text{M}$). The LC cell was identified by its sensitivity to noradrenaline (NA; $100 \mu\text{M}$). The superfusion times of all compounds are indicated by the horizontal bars. The intervals between the traces are shown. Note that recording has two different time-scales.

Figure 5 Effect of noradrenaline on the membrane potential and apparent input resistance of locus coeruleus neurones and interaction with glibenclamide. Constant current was passed through the electrode to hyperpolarize the neurones by about 20 mV. Downward deflections represent electrotonic potentials caused by hyperpolarizing current pulses of constant amplitude. Noradrenaline (NA; $100 \mu\text{M}$) produced a similar hyperpolarization and decrease of input resistance, both before and 10 min after the addition of glibenclamide (Gli; $3 \mu\text{M}$). The superfusion times of all compounds are indicated by the horizontal bars. The intervals between the traces are shown. The pre-noradrenaline membrane potential is indicated by broken lines.

Figure 6 Effect of Ba²⁺ on the membrane potential and firing rate of locus coeruleus neurones, and interaction with noradrenaline and RO 31-6930. Ba²⁺ (2 mM) increased the duration of the action potentials. This improved the reproduction of the spike amplitude by the pen-recorder. Ba²⁺ (2 mM) antagonized the effects of noradrenaline (NA; 100 μ M) and RO 31-6930 (RO; 10 μ M). There is a full recovery of the noradrenaline- (100 μ M) induced hyperpolarization after the washout of Ba²⁺. The intervals between the traces are shown. The superfusion times for all compounds are indicated by the horizontal bars.

of noradrenaline on the membrane potential ($n = 5$).

In 4 further experiments, LC cells were prevented from spontaneously firing by continuous hyperpolarizing current injection through the micro-electrode (Figure 5). In addition, hyperpolarizing current pulses of constant amplitude were delivered at regular intervals in order to measure the input resistance. Noradrenaline (100 μ M) caused a hyperpolarization of 8.6 ± 1.5 mV and decreased the input resistance (127.3 ± 19.7 M Ω) by $27.3 \pm 2.8\%$. After a 10 min presence of glibenclamide (3 μ M) both the noradrenaline- (100 μ M) induced hyperpolarization (8.8 ± 1.4 mV; $P > 0.05$) and the fall in input resistance ($29.9 \pm 1.7\%$; $P > 0.05$) were the same as in the absence of the K_{ATP} channel blocker. Furthermore, in these experiments, glibenclamide (3 μ M) did not alter either the membrane potential (0.6 ± 0.6 mV depolarization; $P > 0.05$) or the input resistance ($11.0 \pm 4.3\%$ increase from 124.5 ± 22.4 M Ω ; $P > 0.05$).

Ba²⁺ (2 mM) prolonged the duration, but failed to influence the amplitude of action potentials; this improved the reproduction of spike amplitudes by the pen-recorder (Figure 6). Immediately after the application of Ba²⁺ the firing rate transiently increased, but returned to its original value afterwards. However, the effects of both RO 31-6930 (10 μ M) and noradrenaline (100 μ M) were abolished in the presence of Ba²⁺ (2 mM) (Figure 6, Table 1).

Discussion

K_{ATP} channels provide a link between the metabolic status and electrophysiological properties of neurones in the central nervous system (Ashcroft & Ashcroft, 1990). This fact has a

number of physiological and pathophysiological implications. Brain neurones are known to be extremely sensitive to hypoxia and reduced supply of glucose, conditions which lead to decreased intracellular ATP concentrations and, in consequence, to opening of K_{ATP} channels (de Weille & Lazdunski, 1990). The resulting hyperpolarization of the cell somata decreases the excitability and conserves energy. Similarly, a possible hyperpolarization of the nerve terminals inhibits transmitter release (Amoroso *et al.*, 1990; Schmid-Antomarchi *et al.*, 1990), and thereby retards metabolic exhaustion. Since hypoxia produces an excessive release of glutamate, activation of $I_{K(ATP)}$ may protect neurones innervated by excitatory amino acid pathways (Ben-Ari, 1989). Finally, in certain areas of the brain (e.g. in hypothalamic nuclei regulating food intake), neurones which possess K_{ATP} channels and respond to hyperglycemia with increased excitability have been described (Ashford *et al.*, 1990).

Various experimental approaches unequivocally document the presence of K_{ATP} channels in the brain. The methods utilized include the measurement of ⁸⁶Rb⁺ efflux, which is an indicator of transmembrane K⁺ movements (Amoroso *et al.*, 1990; Schmid-Antomarchi *et al.*, 1990), the determination of transmitter release (Amoroso *et al.*, 1990; Schmid-Antomarchi *et al.*, 1990) and electrophysiological recordings. The application of ATP to inside-out patches from cerebral cortical (Ashford *et al.*, 1988) and hypothalamic (Ashford *et al.*, 1990) neurones directly demonstrated the subsequent closure of a K⁺ channel. The evidence for the existence of K_{ATP} channels in hippocampal pyramidal cells is less convincing. Apparently, in CA3 cells cromakalim activates an inwardly rectifying K⁺ conductance, which is sensitive to Ba²⁺ but not to tetraethylammonium. It was suggested that

this conductance was different from $I_{K(ATP)}$ (Alzheimer *et al.*, 1989). However, $I_{K(ATP)}$ was reported to be involved in the hyperpolarization of the same neurones by hypoxic episodes (Ben-Ari, 1989), but not by glucose deprivation (Knöpfel *et al.*, 1990). Moreover, the hypoxic depolarization following the hyperpolarization was caused by the release of glutamate from mossy fibres and could be prevented by K_{ATP} channel openers acting at these glutamatergic terminals (Ben-Ari & Lazdunski, 1989; Ben-Ari *et al.*, 1990; Mourre *et al.*, 1991).

We have found in LC cells that two openers of ATP-sensitive K^+ channels depressed the spontaneous discharge of action potentials. The compounds used were cromakalim, a racemic benzopyran derivative, and RO 31-6930, which has a related non-chiral structure of much higher biological potency (Cook, 1988; Edwards & Weston, 1990). In contrast to results in the heart (Sanguinetti *et al.*, 1988; Bott *et al.*, 1992), the duration of action potentials was not decreased in the LC. Moreover, in agreement with findings in cardiac (Sanguinetti *et al.*, 1988; Bott *et al.*, 1992), but not in smooth muscle (Hamilton *et al.*, 1986) or pancreatic β -cells (Henquin & Meissner, 1982; Cook, 1988), the resting membrane potential of LC neurones was not increased by the K_{ATP} channel openers.

Glibenclamide is a sulphonylurea blocker of $I_{K(ATP)}$; tolbutamide is structurally similar, but has a considerably lower biological activity (Schmid-Antomarchi *et al.*, 1987; de Weille & Lazdunski, 1990). In our experiments, tolbutamide given alone markedly increased the firing rate, while glibenclamide only tended to facilitate it. However, both compounds blocked the inhibitory effect of RO 31-6930 on the spontaneous discharge of action potentials. Neither glibenclamide nor tolbutamide depolarized LC cells. These results are analogous with those seen in the heart (Nakaya *et al.*, 1991; Bott *et al.*, 1992) but disagree with findings in pancreatic β -cells (Henquin & Meissner, 1982; Cook, 1988).

We used concentrations of K_{ATP} channel openers and blockers which were previously shown to be effective in cardiac muscle (Ashcroft & Ashcroft, 1990). These compounds altered the firing rate of LC neurones, although they did not change appreciably the membrane potential or the apparent input resistance. The most likely explanation is that the channel openers slightly hyperpolarized the cells whereas the blockers produced a minute depolarization. The small changes of the membrane potential developed over a considerable time, and could not be detected with the high resistance micro-electrodes used by us. In fact, we found that small shifts of the membrane potential by current injection caused immediate alterations of the firing rate, which were similar in their extent to those produced by the potassium channel openers and blockers (see also Williams *et al.*, 1985; Harms *et al.*, 1992).

In LC neurones, TTX markedly depressed the spontaneous discharge of action potentials decreasing the amplitude of the spikes and prolonging their duration. Apparently, after the blockade of sodium conductance by TTX, the cells continued to fire slower calcium action potentials (Illes & Regenold, 1989). The effect of RO 31-6930 persisted after the inhibition of action potential-dependent synaptic inputs by TTX. Thus

it may be concluded that the RO 31-6930-induced depression of the firing rate arises directly at the somata of LC neurones and not indirectly via neighbouring non-LC cells. Unfortunately, we did not succeed in synaptically isolating LC cells by various low Ca^{2+} , high Mg^{2+} media. In these superfusion media, the spontaneous discharge of spikes temporarily increased and subsequently stopped impeding the measurement of RO 31-6930 effects. However, under the conditions of the present study, K_{ATP} channel openers are unlikely to diminish a tonic facilitatory input controlling LC neurones. Such an input derives mostly from the nucleus paragigantocellularis and is mediated by excitatory amino acids (EAA) (Aston-Jones *et al.*, 1991). In brain slices, focal electrical stimulation in the area of LC evokes excitatory postsynaptic potentials which are depressed by the EAA-antagonist kynurenic acid (500 μM) (Cherubini *et al.*, 1988; Williams *et al.*, 1991). Nonetheless, in the absence of electrical stimulation the spontaneous firing rate was not changed by 500 μM kynurenic acid (present study). Hence, a tonic EAA control of the neuronal activity is probably not operative under *in vitro* conditions.

In the substantia nigra, dopamine D_2 -autoreceptors were suggested to be coupled to K_{ATP} channels since the D_2 -receptor agonist quinpirole caused a tolbutamide-reversible hyperpolarization (Roeper *et al.*, 1990; for the opposite view see Hicks & Henderson, 1992). In LC neurones, noradrenaline stimulates α_2 -adrenoceptors, and thereby enhances a potassium permeability with a resulting decrease in firing rate (Egan *et al.*, 1983; Williams *et al.*, 1985). We did not find any antagonism between glibenclamide or tolbutamide on the one hand and noradrenaline on the other, although either blocker of $I_{K(ATP)}$ abolished the effect of RO 31-6930. By contrast Ba^{2+} , which inhibits both the inwardly rectifying K^+ conductance (Constanti & Galvan, 1983; Osmanovic & Shefner, 1987) and the K_{ATP} channels (Quayle *et al.*, 1988; Adams & Nonner, 1990), prevented the inhibitory actions of both noradrenaline and RO 31-6930. These results as a whole indicate that α_2 -autoreceptors of LC cells activate an inwardly rectifying K^+ current (Williams *et al.*, 1988) rather than $I_{K(ATP)}$.

In conclusion, K_{ATP} channels appear to be present on the somata of LC neurones although a presynaptic location on excitatory nerve terminals innervating the LC cannot be definitely excluded. The density of these channels is probably quite low since even relatively high concentrations of cromakalim and RO 31-6930 produce only moderate decreases in the firing rate. Similarly, rather high concentrations of glibenclamide and tolbutamide are needed to increase the spontaneous firing. However, it is possible that $I_{K(ATP)}$ is activated during hypoxia or hypoglycemia. Under these conditions K_{ATP} channels may open and protect LC neurones against further metabolic exhaustion.

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