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Effect of lithium chloride on the neurotransmitter release from adrenergic nerve terminals of guinea-pig atria

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Abstract—Lithium chloride at 1 and 10 mM produced a dose-dependent inhibition of the cardiac response to field stimulation of the adrenergic nerve terminals, without affecting myocardial contractility in electrically stimulated guinea-pig atria. This effect was calcium-independent and was also present in preparations superfused with 10 mM myoinositol. Moreover, 10 mM lithium chloride reduced the positive inotropic effect of tyramine. These results indicate that lithium reduces the evoked release of noradrenaline from the adrenergic nerve endings, probably lowering the content of releasable neurotransmitter.

Lithium is widely used for the treatment of psychotic disorders (Gershon & Shopsin 1973; Schou 1976) although its mechanism of action is not yet clear. However, it has been reported to interfere with the catecholaminergic system and neurotransmission in the central nervous system, and it has been demonstrated that it alters the metabolism of noradrenaline in rat brain, enhancing the destruction of the amine by monoamine oxidases (Schildkraut et al 1966; Corrodi et al 1967; Schanberg et al 1967; Greenspan et al 1970). Noradrenaline turnover is greatly enhanced in rat brain and slightly in the heart by lithium in doses in the therapeutic range (Stern et al 1969; Poitou & Bohuon 1975). An increased noradrenaline uptake by rat isolated synaptosomes has been reported after chronic lithium administration (Colburn et al 1967); on the other hand, acute lithium administration does not modify noradrenaline uptake into rat brain in-vivo (Schanberg et al 1967; Schildkraut et al 1969) or in-vitro (Katz et al 1968).

It has been suggested that lithium treatment induces a reduction in catecholamine release from nerve terminals (Poitou & Bohuon 1975). Katz et al (1968), Katz & Kopin (1969) and Bindler et al (1971) have observed that lithium decreases the release of noradrenaline induced by electrical field stimulation from rat brain slices and cat spleen.

To investigate the effect of lithium on noradrenergic transmission we have used a simpler peripheral model than the central nervous system, i.e. the guinea-pig heart, where noradrenaline release was induced by field stimulation of sympathetic nerve terminals and by the indirect sympathomimetic agent tyramine.

Materials and methods

The methods and apparatus have been previously described (Ledda & Mantelli 1984). Briefly, isolated guinea-pig atria were vertically mounted in a 15 mL glass chamber, containing Tyrode solution of the following composition (mM): NaCl 115; KCl 4·7; $CaCl_2$ 1·8; $MgSO_4$ 1·2; KH_2PO_4 1.2; $NaHCO_3$ 25: glucose 10; the pH of the bathing solution was 7·4 and the temperature was kept constant at 30°C. Tyrode solution was oxygenated with a gas mixture of 97% O_2 and 3% CO_2. A resting tension of 0·8 g was applied to the preparations and maintained throughout the experiments. The preparations were stimulated at a constant rate (4 Hz) through two pointed electrodes. Isometric contractions were recorded by an isometric transducer and a d.c. preamplifier on a pen recorder and a dual beam oscilloscope. The sympathetic nerve terminals were stimulated by field pulses (50 mA amplitude, 1 ms duration), one per consecutive contraction, by means of two platinum plates parallel to the preparations. A control circuit allowed timing of the field pulses to begin 10 ms after the driving pulse, during the absolute refractory period. Stimulus-inotropic response curves were obtained by increasing the number of field pulses from 2 to 12, until the maximum positive inotropic effect was reached. As soon as the contractile tension was returned to the control value, lithium chloride was added and, after 30 min of contact, the stimulus-response curve was repeated.

All the experiments using field stimulation were carried out in the presence of atropine 1 μM, to eliminate the parasympathetic component of the response to field stimulation. Dose-inotropic response curves for noradrenaline and tyramine were obtained by increasing the drug concentration stepwise until the maximum response was reached; then the agonist was removed by washing and, after about 20 min of equilibration, lithium was added and a second curve for the agonists was repeated in the same preparation after 30 min of contact with lithium chloride. Statistical analysis was performed using the paired Student's *t*-test. The drugs used were: atropine sulphate (BDH), lithium chloride (Merk), myoinositol (Sigma), noradrenaline bitartrate (Fluka), tyramine hydrochloride (Sigma).

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Results

Effect of lithium on the sympathetic response to field stimulation. Trains of field pulses applied during the functional refractory period in guinea-pig atria driven at 4 Hz induced a graded positive inotropic effect. Exposure of the preparations to lithium chloride 1 and 10 mM for 30 min produced a dose-dependent reduction of the stimulus-response curve. The effect of 1 mM lithium was slight, even if significant, whereas 10 mM lithium greatly reduced the sympathetic response to field stimulation, especially at the higher stimulation intensities (Fig. 1). Neither of the two lithium concentrations modified the basal contractility of the preparations.

The inhibitory effect of 10 mM lithium on the stimulus-response curve to field stimulation was completely maintained in preparations perfused with Tyrode solution containing increased Ca^{2+} concentrations, i.e. 3·6 and 5·4 mM (not shown in Fig. 1). The effect of the higher lithium concentration (10 mM) was also tested on the dose-effect curve for exogenous noradrenaline to prove that the inhibitory effect of lithium was not due to a postsynaptic effect; it was observed that at 10 mM it did not significantly modify the positive inotropic effect of exogenously-added noradrenaline.

Finally, since it is known that lithium is able to interfere with phosphatidylinositol turnover, thus inducing a reducion of myoinositol availability (Hallcher & Sherman 1980; Sherman et al 1981), some experiments were performed in preparations perfused with Tyrode solution containing 10 mM myoinositol. It was observed in these experiments that 10 mM lithium reduced the inotropic response to field stimulation to the same degree as in the absence of myoinositol.

Effect of lithium on the cardiac response to tyramine. At concentrations between 3 and 100 μM the sympathomimetic agent tyramine induced a positive inotropic effect in guinea-pig isolated atria electrically stimulated at 4 Hz. In previous

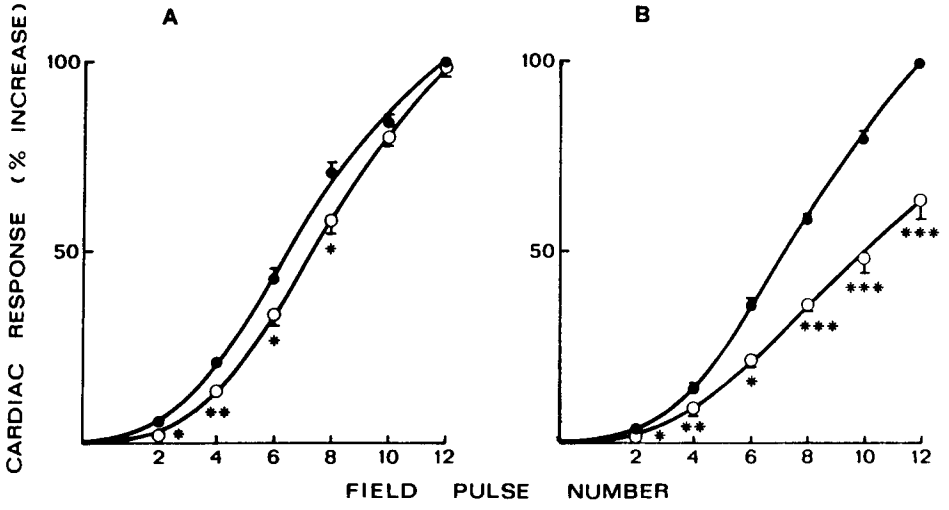

FIG. 1. Effect of lithium chloride on the sympathetic response induced by trains of field pulses in guinea-pig isolated atria stimulated at 4 Hz. Panel A: ● control, ○ lithium 1 mM; panel B: ● control, ○ lithium 10 mM. Means ± s.e.m. of five and six experiments respectively. Statistically significant differences from controls are indicated by the following symbols: * $P<0.05$; ** $P<0.01$; *** $P<0.005$.

experiments it had been observed that a rest period of 50 min was sufficient to obtain a second dose-response curve to tyramine which was perfectly superimposable on the first one. Thus 10 mM lithium was added 20 min after the first concentration-inotropic response curve for tyramine, and the dose-effect curve for the agonist was repeated after a 30 min period of contact with lithium. Fig. 2 shows that 10 mM lithium significantly antagonized the positive inotropic effect of tyramine. The antagonistic effect of lithium versus tyramine was very similar to that obtained using field stimulation.

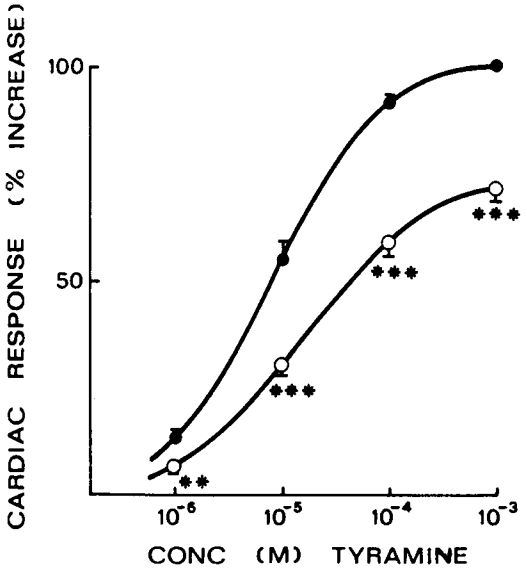

FIG. 2. Effect of lithium chloride on the dose-inotropic effect curve for tyramine in guinea-pig isolated atria stimulated at 4 Hz. ● control, ○ lithium 10 mM. Means ± s.e.m. of six experiments. Statistically significant differences from control are indicated by the following symbols: ** $P<0.01$; *** $P<0.001$.

Discussion

The results of the present study demonstrate that lithium is able to depress the cardiac response to sympathetic nerve stimulation in guinea-pig atria in a dose-dependent manner. This inhibitory effect is attributable to an action at prejunctional level: in fact, even at the higher concentration tested (10 mM), the ion neither modified the cardiac response to exogenous noradrenaline, nor induced any direct depressant effect on cardiac contractility. Since the increase in contractility produced by field stimulation of guinea-pig atria is an index of noradrenaline release (Blinks 1966), these findings confirm that lithium is able to reduce the release of the neurotransmitter from the sympathetic nerve terminals of guinea-pig atria. This conclusion is in good agreement with previous findings obtained in mammalian brain (Katz et al 1968; Katz & Kopin 1969), spleen (Bindler et al 1971) and saphenous vein (Beaty et al 1981). However, according to Katz & Kopin (1969), the inhibition by lithium of the evoked release of noradrenaline in rat cerebral slices is calcium-dependent since it is prevented by the increase of extracellular calcium. However, in our experiments the inhibitory effect of lithium was not reduced by an increase in Ca^{2+} concentration to 3·6 or 5·4 mM. Moreover, it was observed that lithium reduced the positive inotropic response to tyramine, a drug which is able to release noradrenaline by a calcium-insensitive mechanism that differs from that involved in the response to electrical stimulation. Thus, the findings that both tyramine- and electrically-induced noradrenaline release were reduced by lithium, together with the observation that the latter effect is not calcium-sensitive, strongly suggest that, in lithium-pretreated preparations, a reduced amount of releasable noradrenaline is present in the adrenergic nerve terminals. This is in agreement with the observations that in brain lithium enhances the rate of endogenous noradrenaline loss in the presence of a noradrenaline synthesis inhibitor (Corrodi et al 1967), and that the ion increases the metabolism of neural noradrenaline by monoamine oxidases (Schildkraut et al 1966, 1969; Schanberg et al 1967; Stern et al 1969; Greenspan et al 1970).

Our finding that the inhibitory effect of lithium was also present in preparations preloaded with myoinositol rules out the possibility that this effect is linked to an interference with the phosphatidylinositol turnover. It is well-known that lithium interferes with phosphatidylinositol turnover by blocking the activity of the myo-inositol-1-P-phosphatase and reducing the cellular availability of myoinositol, which is needed for the resynthesis of membrane poliphosphoinositides (Hallcher & Sherman 1980; Sherman et al 1981).

Therefore our results, which were obtained in a simple peripheral preparation, demonstrate that lithium reduces the

evoked release of noradrenaline, and suggest that this effect may be due to a diminished content of the neurotransmitter in the nerve endings. Since the blood levels of lithium in patients chronically treated with lithium chloride have been reported to be about 1 mM (Schou 1976), it is suggested that only the slight effect observed with the lower concentration of the ion tested in the present study may be of clinical significance in terms of the mechanism of action of lithium.

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Deamination of aliphatic amines of different chain lengths by rat liver monoamine oxidase A and B

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Abstract—Monoamines with from 1 to 18 straight chain carbon atoms have been analysed as rat liver monoamine oxidase substrates. Methylamine and ethylamine are clearly not substrates of monoamine oxidase (MAO). n-Propylamine, n-butylamine, n-dodecylamine and n-octadecylamine are relatively poor substrates, i.e. with high K_m and low V_{max} values for the enzyme. n-Pentylamine, n-hexylamine, n-heptylamine, n-octylamine, n-nonylamine and n-decylamine are all very good MAO substrates. All these aliphatic amines are found to be typical type B substrates according to the sensitivities of the enzyme towards the selective MAO-B inhibitor selegiline and the MAO-A inhibitor, clorgyline. The sensitivity towards selegiline with respect to these amines is even higher, i.e $K_i = 1 \times 10^{-9}$ M for butylamine, than that of the typical type B substrate β-phenylethylamine ($K_i = 1 \times 10^{-8}$ M). The sensitivity towards selegiline decreases slightly with increasing chain length of these aliphatic amines.

Monoamine oxidase (MAO, EC 1.4.3.4.) is well known for its catalytic activities on endogenous aromatic monoamine substrates, such as neuronal catecholamines, indoleamines and trace amines (Blaschko 1974). The enzyme is also responsible for the detoxification of xenobiotic amines. Two types of enzymes, namely MAO-A and MAO-B, are classified according to substrate preference (i.e. type A substrate 5-hydroxytryptamine and type B substrate β-phenylethylamine) and to sensitivity towards selective MAO inhibitors, such as clorgyline (MAO-A inhibitor) and selegiline (L-deprenyl) (MAO-B inhibitor) (Fowler et al 1978; Denney & Denney 1985). It has been shown earlier in man that after administration of short chain aliphatic amines methylamine was totally metabolized and excreted as urea, whilst about one-third of ethylamine and less than 10% of propylamine and n-butylamine were recovered unchanged in the urine (Rechenberger 1940). It was later found by Blaschko (1952) that short chain aliphatic amines can be oxidized by rabbit liver MAO, and by von Korff & Wolfe (1984) that monoamines of 8 to 12 carbon atoms were also substrates for beef liver MAO. These latter amines can act as time-dependent reversible MAO inhibitors. n-Pentylamine has been found to be deaminated by MAO-A and B, as well as by semicarbazide sensitive amine oxidase in the rat heart (Guffroy et al 1983).