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AN EXCITATORY ACTION OF IONTOPHORETICALLY ADMINISTERED LITHIUM ON MAMMALIAN CENTRAL NEURONES

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1 The action of iontophoretically administered lithium was studied on spinal Renshaw cells and interneurones and on supraspinal neurones in cerebral cortex, thalamus, hypothalamus and brain stem in anaesthetized cats and rats.

2 There was a correlation between the effects of Li^+ and those of acetylcholine (ACh), although rather more cells were unaffected by Li^+ than by ACh.

3 The usual effect was an excitation of rather slow onset, but occasionally effects were produced with time courses similar to those of ACh. The excitation was blocked by ACh antagonists and was best demonstrated with dihydro-β-erythroidine on Renshaw cells.

4 The postsynaptic excitant action of ACh on Renshaw cells was reduced by Li^+.

5 Depressant actions of Li^+ were encountered on cells also depressed by ACh.

6 It is concluded that Li^+ may facilitate cholinergic transmission at some sites in the CNS by increasing the release of ACh by an unknown mechanism. Similar effects at non-cholinergic synapses might also occur but would appear to be of less importance. Since facilitation of neuronal firing with Li^+ was usually observed, the depressant effects on postsynaptic responses to ACh may be of little consequence.

Introduction

In recent years, lithium salts have come into prominence in the management of affective discorders, especially in the treatment of manic-depressive psychosis. Despite many investigations, the mechanism by which lithium exerts its therapeutic effects remains obscure (for recent reviews and references see Gershon & Shopsin, 1973; Johnson, 1975; Bunney & Murphy, 1976). One difficulty is that therapeutic effects are obtained at low plasma levels of 0.8–1.5 mequiv/l and only after repeated administration for at least several days (Coppen, 1973). The delay in onset of the therapeutic effect may be due to the fact that this plasma level, which is the maximum that can be tolerated without untoward side-effect, cannot rapidly build up an effective concentration at a central site of action. This difficulty might be overcome by administration of lithium directly at its site of action on central neurones. In this respect, the iontophoretic method used in this study represents the best technique currently available, but suffers from the disadvantage that it may only examine acute, rather than long term effects (Segal, 1974). However, if the therapeutic delay is only due to the slow kinetics of accumulation of lithium at central sites, then this disadvantage of the electrophoretic technique may not be important.

In the majority of *in vitro* studies rather high concentrations of lithium have been employed and these may be expected to produce nonspecific actions on excitable membranes by virtue of the fact that lithium substitutes for sodium in diffusion across cell membranes but is not handled effectively by the sodium pump. In one exceptional study with low concentrations of 1–5 mM (Ploeger & den Hertog, 1973), it was shown that lithium affected the activity of the sodium-potassium pump in isolated nerve fibres, probably by competition between lithium and potassium ions.

Such effects of lithium on ionic mechanisms are unlikely to affect central neurones in a specific fashion unless some aspects of the transmission process, or particular central neurotransmitters are more dependent upon a particular ionic process than others. Thus, it is not impossible to reconcile such a general basic mechanism of action with a modification of central neurotransmission specific enough to explain the rather selective therapeutic action of lithium and there is a great deal of mainly biochemical evidence showing changes in the levels, turnover or release of certain putative transmitters.

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Our interest in a possible link between the therapeutic action of lithium and cholinergic mechanisms arose from observations by Lee, Lingsch, Lyle & Martin (1974) and Lingsch & Martin (1976) that choline uptake into human erythrocytes is greatly reduced in lithium-treated patients and that the effect seemed to be irreversible and independent of the presence of lithium at the time of measurement. It seemed possible that a similar process occurring in central cholinergic terminals might reduce acetylcholine (ACh) synthesis, thereby impairing cholinergic transmission.

Although this working hypothesis was not substantiated by the present investigation, evidence was obtained which indicated that lithium probably does modify central cholinergic transmission through a presynaptic action, although the effect is probably not absolutely specific for cholinergic terminals.

A preliminary report of this study was presented to the British Pharmacological Society (Haas & Ryall, 1974).

Methods

This study was carried out on 207 neurones in 40 cats and 21 neurones in 7 rats. Cats were anaesthetized either with pentobarbitone sodium (35 mg/kg i.p.) or with chloralose (60 mg/kg i.v.) after halothane induction and anaesthesia was supplemented when required by intravenous injection. A few experiments were performed under continuous halothane anaesthesia. Rats were anaesthetized with a mixture of urethane and pentobarbitone (400 mg/kg plus 5 mg/kg i.p.) supplemented by pentobarbitone when required.

In experiments on spinal neurones, the cord was exposed by a laminectomy extending from L2 to L7 and ventral roots S1 and L7 were dissected free and mounted upon bipolar electrodes for stimulation in order to identify Renshaw cells by their characteristic discharge to antidromic volleys in ventral roots. Interneurones were located by their response to stimulation of a dorsal root or a peripheral nerve in the ipsilateral hind limb; they were not excited by antidromic volleys in ventral roots.

The head of the animal was fixed in a stereotaxic frame for location of supraspinal neurones. Cortical neurones were located in the pericruciate region but were not further identified. Thalamic neurones were located by stereotaxic coordinates and some were identified as presumed relay neurones if they were excited at short latencies on stimulation of the median nerve or medial lemniscus. A few were also identified by antidromic invasion from the cerebral cortex. Hypothalamic neurones were approached stereotactically from the dorsal side (cats) or transpharyngeally in rats (Dreifuss & Kelly, 1972) and some were identified as neurosecretory neurones by antidromic invasion from the hypophyseal stalk.

Extracellular records were obtained via the centre barrel of multi-barrelled micropipettes with overall tip diameters of 5–8 μm or via a projecting barrel of a parallel micropipette assembly. Recording barrels were filled with 4 M NaCl. After suitable amplification and discrimination of single units, standard pulses were led into an R–C ratemeter or a digital counter. The input to the counter could be gated so that action potentials evoked in response to nerve stimulation could be counted for a variable time after the stimulus, thus avoiding counting background 'spontaneous' firing of the cell. The counter was reset only after many (10–50) stimulus presentations. The chart record therefore continuously displayed either mean firing frequency or average number of discharges after a stimulus.

Exposed spinal cord was covered by a pool of warmed paraffin oil and the animal was maintained at about 37–38°C by heating pads automatically controlled by a thermistor-operated circuit.

Outer drug containing barrels of the micropipettes contained LiCl (1 M), ACh bromide (0.5 M), atropine sulphate (0.01 M in 0.16 M NaCl), dihydro-β-erythroidine (DHBE, 0.02 M in 0.16 M NaCl), NaCl (4 M) or, occasionally, L-glutamic or DL-homocysteic acid (Na salt, 0.2 M, pH 8). Retaining voltages of 0.5 V were routinely applied to drug-containing barrels. Typical electrode resistances ranged from 5–10 MΩ for recording barrels and 20–100 MΩ for drug-containing barrels.

Results

The effect of lithium on Renshaw cells

Lithium was administered with electrophoretic currents up to 400×10^{-9} A to 39 Renshaw cells in 10 cats, while continuously observing discharges to submaximal (18 cells) or maximal (2 cells) ventral root stimulation, or background, 'spontaneous' firing (20 cells) or responses to periodic iontophoretic administration of ACh (10 cells). Submaximal stimulation of ventral roots produced rather variable discharges and these were counted and automatically averaged for a number of repetitions and continuously plotted on a pen recorder; submaximal rather than maximal stimulation was employed initially in anticipation of an expected decrease in the response but was continued in later experiments when it became apparent that the effects of Li^+ were more likely to be excitatory than depressant. On some cells combinations of these techniques were employed.

Excitatory effects of Li^+ were encountered in 24 of 39 cells examined. The characteristics of the change in excitability varied from cell to cell and even sometimes changed on repeated administration to the same cell. This variability in the response presented a major difficulty in analysing the mechanism of the excitatory

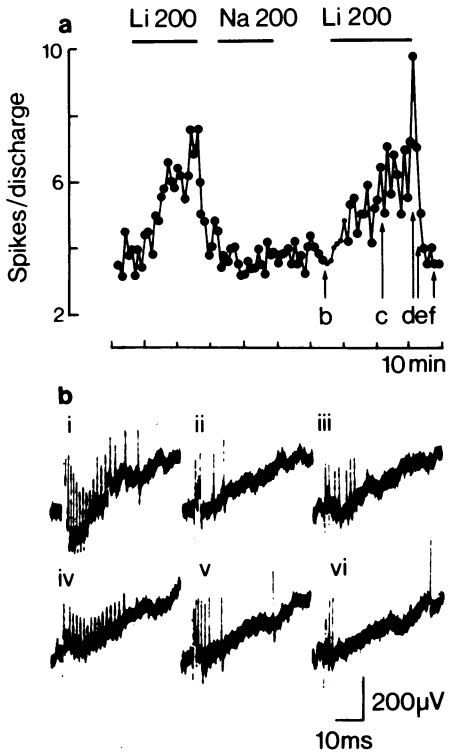

Figure 1 (a) Effect of iontophoresis of Li^+ (200×10^{-9} A) or Na^+ on the submaximal discharge of a Renshaw cell evoked by ventral root stimulation. Each point is the average of 10 discharges.Ordinate scale: average number of spikes per discharge. Abscissa scale; intervals of 10 minutes. (b) A sample photograph of a maximal discharge in (i) and sample records of submaximal discharges before (ii), during (iii, iv) and after (v, vi) Li^+ iontophoresis at the times indicated in (a). Calibrations 200 μV and 10 milliseconds. Photographs taken on film moving along the vertical axis.

effect, particularly when using antagonists, and successful experiments with antagonists were only possible on relatively few occasions.

The most usual effect encountered was a very slow increase in either the average rate of background discharge (in 10 of 20 cells tested) or in the number of action potentials evoked by a submaximal ventral root volley (in 13 of 18 cells tested). The electrophoretic currents employed usually ranged from 100 to 300×10^{-9} A. The evaluation of smaller currents was usually precluded by the variability in the response with repeated applications but on the few occasions when they were tested they were less effective.

One of the earliest signs that Li^+ would ultimately produce an effect was often that action potentials began to occur in bursts. As the excitatory effect progressed so the repetition rate of the bursts and the frequency within the bursts tended to increase.

Figure 1 illustrates a typical effect of 200×10^{-9} A Li^+ on the discharge of a Renshaw cell evoked by submaximal stimulation of L7 ventral root. Each point in Figure 1a is the average of 10 repeated stimuli. In the absence of Li^+ there was an average of about four spikes in each discharge, compared with 14 spikes evoked by maximal antidromic volleys (Figure 1b(i)). During the administration of Li^+ the magnitude of the evoked discharge increased slowly over a period of about 20 min and returned to control values when Li^+ was discontinued. The effect on this cell was reproduced by a second administration. The effects were not considered to be due to the currents themselves for several reasons. The effects were not reproduced by the administration of Na^+ ions with the same electrophoretic current, nor were they counteracted by the simultaneous administration of a 'balancing' current ejecting Cl^- from another barrel (not illustrated). Finally, the slow onset of the effect renders it unlikely that the excitation was due to the current *per se*.

A reduction in spike amplitude was often seen during the high frequency firing evoked by Li^+ and in the discharges evoked trans-synaptically during intense excitation by Li^+ (Figure 1b(iv)). No particular significance can be attached to this observation because Renshaw cells tend to show spike inactivation during high frequency firing (see control record in Figure 1b(i)), regardless of the method used to evoke such firing.

An effect similar to that in Figure 1 is also shown in Figure 2a on another cell. On this cell (Figure 2b,c) the effect on background activity is also illustrated. It may be seen that, during and after Li^+ administration, the variability in the background activity was increased due to the occurrence of 'burst' activity (Figure 2b).

Reproducible excitatory effects on background activity were seen on only a few occasions. An example is shown in Figure 4f in which Li^+ produced an excitation similar to that produced by ACh. On other cells such an excitation was observed only during the first administration of Li^+ and was either much reduced or absent subsequently. Transient 'burst-like' firing was seen on 3 cells, but on one of these it was only apparent a short while after the administration of ACh (Figure 3c) for a few administrations of Li^+. An extreme example of 'burst-like' firing is shown in Figure 4a,b. On some cells the increase in burst firing did not recover within the period of observation after cessation of Li^+ administration.

Thus Li^+ caused an increase in synaptically evoked discharges and background activity with a variable time-course and reproducibility which did not seem to be due to current artefacts. Such changes could have

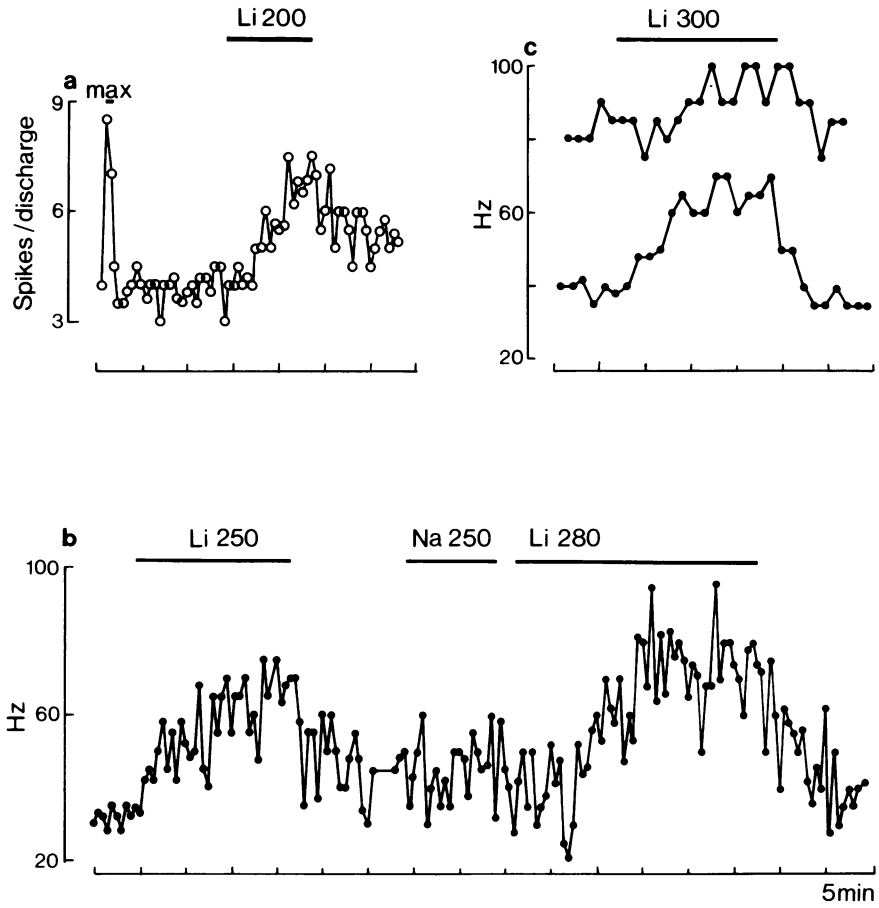

Figure 2 Effects of Li^+ iontophoresis on a Renshaw cell. The average of 10 discharges to a brief period of maximal stimulation (max.) of the ventral root and, subsequently, repeated submaximal activation at 10 Hz is shown in (a); (b) and (c) show the action of Li^+ and Na^+ on background activity (b and lower record in c) and on the maximum frequency of firing attained during the repeated iontophoresis of acetylcholine (ACh) with a current of 8×10^{-9} A (upper record in c). Despite the slight increase in maximal firing frequency to ACh (c) the elevation in firing frequency above the background rate (the difference between the upper and lower records in c) was reduced. Ordinate scales; average number of action potentials per discharge in (a), frequency of firing (Hz) in (b) and (c). Abscissa scale; 5 min intervals.

been produced by either pre- or postsynaptic effects. The effects of Li^+ on the firing evoked by ACh was therefore tested on 10 cells on the assumption that ACh causes firing by an action on postsynaptic receptors.

On 2 cells there was no change in the response to ACh. On another (Figure 3a) there was an increase in the background discharge and a slight reduction in the increase in frequency caused by ACh although there was a slight elevation in the maximum firing frequency. On the remaining cells, the effect of ACh was reduced, even in the absence of changes in background activity (Figure 3b).

It therefore seemed possible that Li^+ might excite Renshaw cells by causing a release of transmitter from presynaptic terminals. The only identified excitatory transmitter on Renshaw cells is ACh and dihydro-β-erythroidine (DHBE) is an ACh antagonist at the nicotinic receptors (Curtis & Ryall, 1966a, b). DHBE was therefore tested by iontophoresis on 4 cells on which the excitatory effect of Li^+ was either repeatable or maintained during the administration. On all 4 cells the excitatory effect of both ACh and Li^+ was blocked by DHBE (Figure 4b, f). Some recovery of the Li^+ effect is evident in Figure 4f but the cell in (b) was lost before recovery from DHBE was observed.

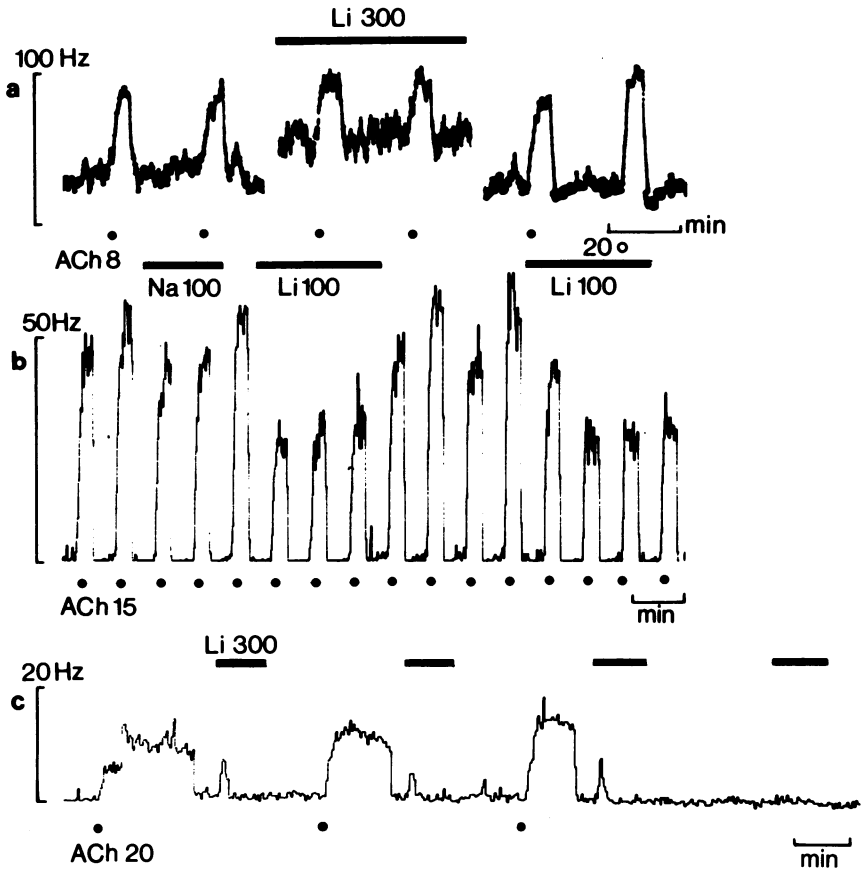

Figure 3 Interaction between Li^+ and acetylcholine (ACh) on Renshaw cells. Data obtained on 3 different cells. (a) Li^+, 300×10^{-9} A elevated background firing rate with only a small reduction in the increased firing frequency produced by ACh, 8×10^{-9} A. (b) Li^+, 100×10^{-9} A decreased ACh-evoked responses with no increase in background activity: Na^+, 100×10^{-9} A had no clear effect. (c) Li^+, 300×10^{-9} A caused transient bursts of firing only when administered a short time after the administration of ACh. Calibrations; firing frequency in Hz and minutes. Administration of Li^+ is indicated by the horizontal bars above the records. ACh (●) was administered for periods of 20 s, (a, b) or 60–100 s (c).

The effect of hemicholinium on Renshaw cells

The original rationale for the lithium experiments was based upon a possible hemicholinium (HC-3)-like action in blocking the uptake of choline (see Introduction). HC-3 was therefore tested upon 13 Renshaw cells. On 4 cells diffusion of HC-3 from the micropipette, achieved by removing the retaining current, caused a gradual decline in the number of spikes elicited in each discharge to ventral root stimulation and it was observed that even in the presence of the retaining current on 2 cells there was a gradual decline in the evoked response after first locating the neurone. On 1 cell currents of 15–70 nA caused no apparent change in the evoked response. On the remaining 8 cells hemicholinium caused a mixture of depressant and excitatory effects. The depression was seen usually as an increased latency of the first spike in the evoked response (Figure 5b–d), sometimes accompanied by a small decrease in the maximal evoked response. The excitation was revealed as a marked increase in the number of discharges in an evoked submaximal response (Figure 5b–e). The excitatory effect was usually the most obvious action of HC-3 and tended to be fast in onset, although on one cell a slow time course was observed, similar to that observed with Li^+.

The increased latency to the first action potential after HC-3 administration in the presence of facilitation of submaximal discharges is difficult, if not

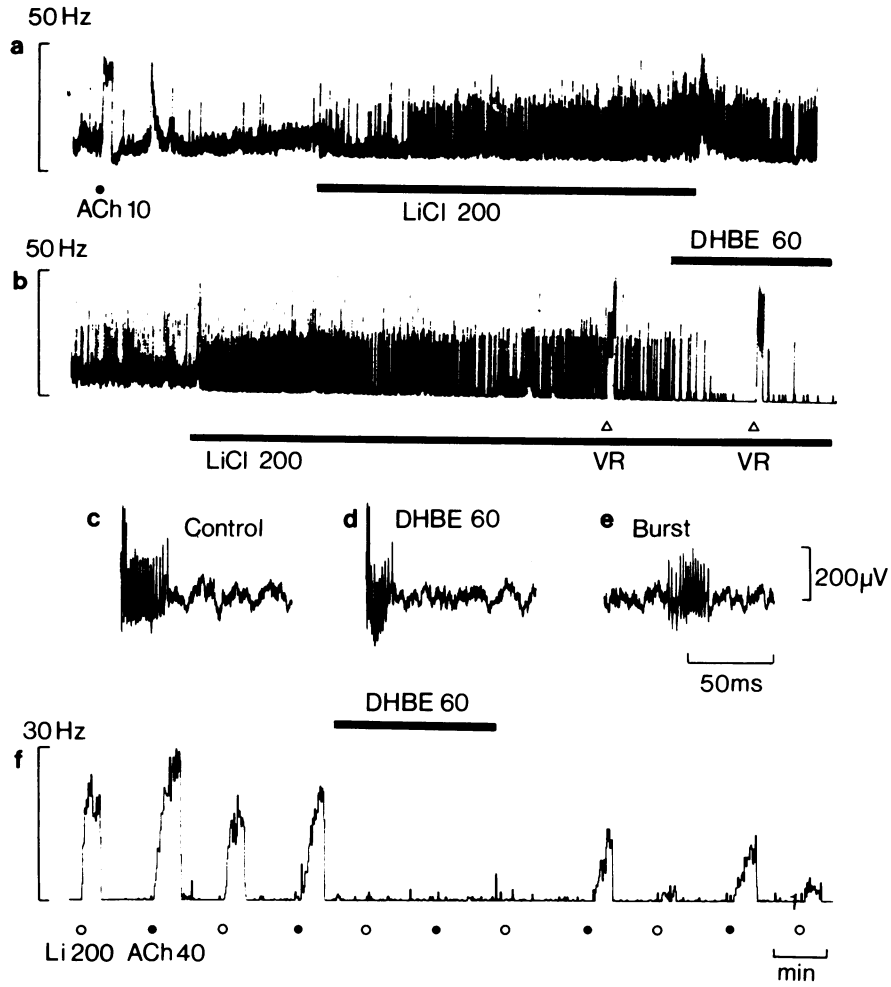

Figure 4 Interaction between excitation by Li^+ and dihydro-β-erythroidine (DHBE) on two Renshaw cells (a–e and f). The cell in (a) was excited by acetylcholine (ACh) 10×10^{-9} A. Li^+, 200×10^{-9}, compensated by the simultaneous ejection of Cl^- from another barrel, caused the appearance of 'burst'-like firing which increased gradually and only slowly waned after the cessation of Li^+ administration. A typical record of a single 'burst' is shown in (e). The effect was repeatable with a second administration of Li^+ in (b) and the 'bursts' were abolished by the administration of DHBE (60×10^{-9} A), which reduced the maximal discharge to antidromic ventral root (VR) stimulation (c and d). (f) Firing produced by Li^+ (O) on this cell was similar to that produced by ACh (●) and was unusually fast in onset and repeatable. DHBE (60×10^{-9} A) abolished responses to both ACh and Li^+, with partial recovery after the termination of the DHBE ejecting current. Calibrations: firing frequency in Hz and min in (a), (b) and (f); 200 μV and 50 ms in (c–e). Duration of Li^+ administration indicated by horizontal bars below records in (a) and (b) and of DHBE administration by bars above the records in (b) and (f). Four min interval between records (a) and (b).

impossible to interpret because the former probably represents a presynaptic and the latter a postsynaptic action. The postsynaptic nature of the facilitation is indicated, but not proved, by the excitation of non-cholinoceptive interneurones.

In general, the effects of HC-3 on Renshaw cells confirm the observations by Quastel & Curtis (1965).

Comparison of actions of Li^+ and hemicholinium on interneurones

Lithium, administered with electrophoretic currents of $300–400 \times 10^{-9}$ A failed to excite any of the 6 spinal interneurones on which it was tested. ACh was also ineffective on these cells. In contrast, HC-3

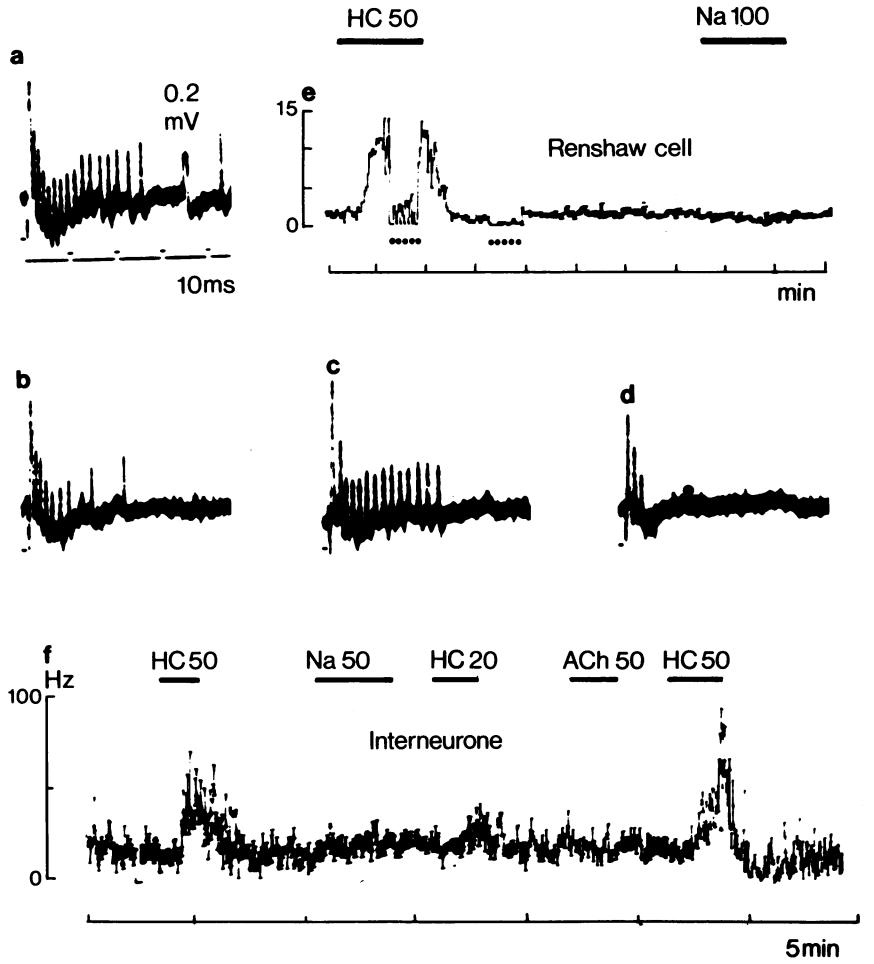

Figure 5 The action of hemicholinium (HC-3) on a Renshaw cell (a–e) and an interneurone (f). Records of a synaptically evoked discharge evoked by maximal (a) and submaximal stimulation of the ventral root before (b), during (c) and after (d) the iontophoretic administration of HC-3 with 50×10^{-9} A. Calibrations for (a–d) are shown in (a). The trace in (e) shows the average number of spikes in each submaximal discharge to a ventral root volley. During the period indicated by ..... the rate of stimulation was reduced from 5 Hz to 1 Hz, resulting in fewer counts in each counting epoch; records (c) and (d) were obtained at these times. A control current of 100×10^{-9} A ejecting Na^+ was ineffective. Time intervals on the abscissa scale are in minutes. HC-3 ($20–50 \times 10^{-9}$ A) also increased the 'spontaneous' background discharge of an interneurone (f) which was insensitive to ACh (50×10^{-9} A) and Na^+ (50×10^{-9} A). Ordinate scale: average firing frequency in Hz. Abscissa scale: time intervals are 5 minutes.

(20–100 nA) excited all 4 interneurones on which it was tested (Figure 5f). Thus the excitatory action of Li^+ appeared to be selective for Renshaw cells, whereas the excitation by HC-3 was not.

The effect of lithium on supraspinal neurones

Table 1 shows an analysis of the data obtained on 157 supraspinal neurones in 21 cats and 7 rats.

A total of 123 cells was excited by ACh, 9 were inhibited and 25 were unaffected. Lithium excited 85 neurones, inhibited 7 and had no effect on the remainder.

There was a close correlation between the effect of ACh and that of Li^+. Lithium and acetylcholine had similar effects on 91 cells, and inhibition by Li^+ was only observed on thalamic neurones, which were the only cells on which depression by ACh was noted. On

Table 1 A comparison of the action of Li^+ and acetylcholine (ACh) on 157 supraspinal neurones in cats and rats

Site	*Effect of ACh*	*Number of cells affected by Li^+*			*Sub-totals*	*Totals*
		+	—	0		
Thalamus (12 cats)	+	34	4	41	79	
	—	4	3	2	9	
	0	4	0	6	10	98
Thalamus (3 rats)	+	3+1*	0	0	4	
	—	0	0	0	0	
	0	1	0	3	4	8
Pericruciate cortex (3 cats)	+	8	0	3	11	
	—	0	0	0	0	
	0	1	0	1	2	13
Pericruciate cortex (2 rats)	+	6	0	0	6	
	—	0	0	0	0	
	0	0	0	0	0	6
Hypothalamus (13 cells in 3 cats and 7 cells in 2 rats)	+	13	0	0	13	
	—	0	0	0	0	
	0	1	0	6	7	20
Brain-stem (3 cats)	+	7	0	3	10	
	—	0	0	0	0	
	0	2	0	0	2	12
Totals		85	7	65	157	157

*ACh was not tested on this cell.
+, excitation; —, inhibition; 0, no effect.

Table 2 Effect of iontophoretic or intravenous atropine on excitation of cortical and thalamic neurones in 7 cats and 2 rats

	Effect of atropine (Atr)*						
Agonist	*a*	*b*	*c*	*d*	*e*	*f*	
ACh	NT	—	—	—	—	—	
Li+	—	—	0	—	—	NT	
Glut or DLH	NT	NT	NT	—	0	0	
Atagonist	*Number of cells*						*Totals*
Atr 10–50 × 10^{-9} A	4	5	4	2	1	3	19
Atr 0.5 mg/kg i.v.	0	2	0	0	0	0	2

*Antagonism of excitation by atropine (Atr) is indicated (—); no effect is indicated (0); NT indicates that a particular agonist was not tested on these cells.
Glut, L-glutamic acid; DLH, DL-homocysteic acid.

only 8 neurones did one agent excite while the other inhibited and all of these were in the thalamus.

The time course of excitation by Li^+ was often, but not invariably, faster than that observed on Renshaw cells but effects of Li^+ and, to a lesser extent, of ACh were variable when tested repeatedly on the same cell.

The effect of atropine on excitation of supraspinal neurones

Iontophoretic and, in two cats, intravenous administration of atropine on responses to ACh, Li^+ and excitant amino acids was examined in neurones in the cerebral cortex and thalamus of cats and rats (Table 2). The variability in the responses to Li^+ precluded many successful tests. In order to ascertain whether the effects of atropine were specific for ACh receptors we would have wished to have tested the effects of atropine on responses to ACh, Li^+ and an excitant amino acid on the same cell, but this was rarely possible, despite intensive efforts. Table 2 shows that iontophoretic atropine reduced the effect of ACh in the 15 cells tested and reduced the excitant effect of Li^+ on 12 of the 16 neurones examined. On 4 neurones, the action of atropine was selective for ACh receptors, leaving responses to excitant amino acids unchanged (Table 2, columns e, f), but on 2 cells (Table 2, column d) atropine did not have a selective action.

Discussion

These experiments have failed to substantiate our original working hypothesis that lithium ions might impair cholinergic transmission by blocking the uptake of choline into cholinergic terminals in the central nervous system. Instead, our study indicated that cholinergic transmission is facilitated, rather than impaired.

The facilitation of cholinergic transmission was demonstrated on the Renshaw cell where Li^+ not only facilitated transmission at the cholinergic synapses of motor axon collaterals but also elevated the background discharge rate known to be blocked by the acetylcholine antagonist atropine (Curtis & Ryall, 1966b). The fact that the discharge evoked by the iontophoretic administration of ACh was usually reduced indicates that the facilitation of transmission was due to a presynaptic action causing an increased release of excitatory transmitter. Similar depressant effects of Li^+ on ACh responses have been demonstrated on mammalian sympathetic ganglion cells (Pappano & Volle, 1967) and on ganglion cells in *Aplysia* (Ono, Sato & Muruhashi, 1974). The depressant effects could be due to a postsynaptic membrane stabilizing action brought about by an increased potassium conductance (Partidge & Thomas, 1974). However, in the rat hippocampus (Segal, 1974) iontophoretic Li^+ did not antagonize the excitatory effect of ACh but reduced the inhibitory action of noradrenaline and 5-hydroxytryptamine but not that of γ-aminobutyric acid.

Dihydro-β-erythroidine is a potent and selective ACh antagonist at postsynaptic nicotinic receptors on Renshaw cells (Curtis & Ryall, 1966a,b). It reduced not only the synaptically evoked discharges and responses to ACh but also reduced the discharges caused by Li^+. It therefore seems reasonable to propose that a part, at least, of the facilitatory action of Li^+ on Renshaw cells was attributable to a presynaptic mechanism causing the release of the endogenous transmitter ACh. The time course of the facilitation by Li^+ was often although not invariably slow, which would be consonant with a slow intracellular accumulation of Li^+ within the terminals. This slow build-up might account for the variability in the effects observed on different cells and might be critically dependent upon the precise location of the ejecting micropipette. A presynaptic effect, causing the release of ACh, is in agreement with observations made at the neuromuscular junction (Kelly, 1968; Carmody & Gage, 1973; Crawford, 1975) and on resting release of ACh from rat cortical slices (Vizi, Illés, Rónai & Knoll, 1972).

However, it is difficult to conceive a mechanism by which Li^+ would have an effect on presynaptic and not on postsynaptic structures. The fact that responses to ACh were reduced by Li^+ supports this postulate. However, it must be concluded that the facilitation exerted on presynaptic structures must more than counterbalance the depressant effect observed postsynaptically, possibly due to a greater dependence of the release mechanism on small ionic changes brought about by the accumulation of Li^+.

A question which arose from these observations was whether Li^+ might have a similar effect on all presynaptic terminals, including non-cholinergic as well as cholinergic terminals. Since Li^+ did not excite spinal interneurones, which were not cholinoceptive and therefore presumably were not innervated by cholinergic terminals, the evidence is suggestive that Li^+ has a predilection for cholinergic rather than non-cholinergic terminals.

The mixed inhibitory-excitatory action of hemicholinium on Renshaw cells has been reported previously (Quastel & Curtis, 1965), but the fact that it also excites non-cholinoceptive interneurones indicates that the excitatory action has no relation to cholinergic transmission.

The predilection of Li^+ for cholinergic synapses in the central nervous system is also supported by the observations made on supraspinal neurones in the cerebral cortex, thalamus, hypothalamus and brainstem. At these sites an excitatory action of Li^+ was usually associated with an excitatory action of ACh, although the reverse situation was less evident, possibly due to a lower efficacy of Li^+ compared

with ACh. On a few cells in the thalamus, probably located in the nucleus reticularis (Ben-Ari, Dingledine, Kanazawa & Kelly, 1976) a depressant effect of both Li^+ and ACh was observed, again supporting the postulated relationship between the action of Li^+ and cholinergic synapses.

Although relatively few in number, there were some instances of a lack of correlation between the action of Li^+ and ACh at supraspinal levels and we must not be trapped into the probably false conclusion that Li^+ only affects cholinergic synapses in the central nervous system. It seems unlikely that any mechanism could be postulated by which such an absolute specificity could occur and there are numerous reports in the literature of changes in other neurotransmitter systems after Li^+ administration. However, our results do indicate a degree of specificity at cholinergic locations and it is possible that alterations in the function of other neurotransmitter systems may arise secondarily from changes in cholinergic transmission. It is unlikely that the effects observed in the present series of experiments were due to secondary effects on other transmitters because the iontophoretic technique ensures a degree of localization of the effects not attainable by systemic administration.

The antagonism of the effects of Li^+ on supraspinal neurones by atropine supports the conclusion that they were often mediated via a release of ACh.

Ba^{2+} selectively excites cholinoceptive neurones in the cerebral cortex (Krnjević, Pumain & Renaud, 1971). However, this effect is claimed to be postsynaptic because it was not blocked by atropine and therefore appears to be different from that of Li^+ in the present experiments. However, Ba^{2+} stimulates the secretion of catecholamines from the adrenal medulla by a mechanism which differs from that of ACh in being independent of external Ca^{2+} (Douglas & Rubin, 1964), and release of ACh by Li^+ at the neuromuscular junction can also occur in the absence of external Ca^{2+} (Carmody & Gage, 1973). It is therefore possible that the presumed effects of Li^+ on ACh release in the present investigation may be similar to that of Li^+ at the neuromuscular junction and to that of Ba^{2+} on catecholamine release in the adrenal medulla.

In conclusion, it is believed that this study indicates that the central actions of lithium may be chiefly due to a facilitating effect on transmitter release, although there was also some evidence of a postsynaptic depressant action on cell excitability of less importance. It is also concluded that the presynaptic effect may be more pronounced on cholinergic than non-cholinergic terminals. It is suggested, tentatively, that this mechanism could be the basis of the therapeutic efficacy of Li^+ in the treatment of manic-depression. In this condition, the slow onset of the effect of Li^+ could be attributable to a slow accumulation of Li^+ within the terminals, limited by the low plasma levels that can be tolerated. However, the occurrence of a relatively non-reversible mechanism (Lingsch & Martin, 1976) which does not depend on relatively simple ionic disturbances cannot be discounted in the explanation both of the therapeutic effect of Li^+ and the postulated facilitation of transmitter release.

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