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THE EFFECT OF LITHIUM ON THE INCREASE IN FOREBRAIN 5-HYDROXYINDOLEACETIC ACID PRODUCED BY RAPHE STIMULATION

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1 The change in forebrain 5-hydroxyindoleacetic acid (5-HIAA) concentration induced by raphe stimulation has been studied in rats treated with Li^+ or 0.9% w/v NaCl solution (saline) for 10 days.

2 Raphe stimulation increased the forebrain concentration of 5-HIAA in both groups of animals. Chlorimipramine abolished this effect in the control group, but not in the Li^+ group.

3 The inhibition of 5-hydroxytryptamine (5-HT) uptake by chlorimipramine was not affected by pretreatment with Li^+ or by the addition of Li^+ to synaptosomal suspensions *in vitro*.

4 It is suggested that the production of 5-HIAA following raphe stimulation in Li^+-treated animals is derived from the metabolism of 5-HT which remains within the intracellular environment. The consequence of this in relation to transmitter release is discussed.

Introduction

Activation of ascending 5-hydroxytryptamine (5-HT) pathways in rat brain by electrical stimulation of the nucleus raphe medianus increases the forebrain concentration of 5-hydroxyindoleacetic acid (5-HIAA) the major metabolite of 5-HT (Aghajanian, Rosecrans & Sheard, 1967; Kostowski, Giacolone, Garattini & Valzelli, 1969). Treatment with imipramine or chlorimipramine significantly reduced the increase of 5-HIAA induced by raphe stimulation, probably by inhibiting 5-HT uptake and thereby denying extracellularly released 5-HT access to intraneuronal monoamine oxidase (Samanin, Ghezzi & Garattini, 1972; Collard & Roberts, 1974). These findings indicate that a large proportion of the 5-HIAA produced by raphe stimulation may arise from the metabolism of 5-HT which had been released extraneuronally and then transported into cells for subsequent metabolism. The elevation of 5-HIAA concentration produced by raphe stimulation therefore may have a use as a measure of the release of 5-HT *in vivo*.

Previous investigations of the effect of lithium (Li^+) on the release of 5-HT from rat brain slices *in vitro* have provided conflicting reports (Katz & Kopin, 1969; Saldate & Orrego, 1975). In the present study, the increase in 5-HIAA following raphe stimulation has been used to examine the effect of Li^+ on the release of 5-HT in the rat forebrain *in vivo*. In addition, a number of studies were also conducted in order to clarify the interpretation of the data obtained in the raphe stimulation study.

A preliminary account of this work has been published (Collard, 1976).

Methods

Raphe stimulation study

Two groups of 36 male Albino Wistar rats (weight 150–250 g) were used in this study. Animals were housed in groups of 4 per cage and give free access to food (Rodent breeding diet, Spratts Patent Ltd.) and water. Each animal in one group received an intraperitoneal injection of LiCl (0.75 mmol/kg) each day for 10 days whilst those in the other group received 0.9% w/v NaCl solution (saline); 24 h after the final injection of Li^+ or saline, half of the animals in each group received chlorimipramine (5 mg/kg i.p.), while the others received an equivalent volume of saline. Three hours after the injection of chlorimipramine or saline, pairs of animals from each of the 4 subgroups (saline alone, saline plus chlorimipramine, Li^+ alone and Li^+ plus chlorimipramine) were anaesthetized with 1% fluothane in O_2 and a stainless steel bipolar electrode (500 μm diameter, tip separation 1 mm) was stereotaxically positioned in the nucleus

raphe medianus according to the following coordinates, lateral 0; anterio-posterior +0.4 mm; vertical −2.6 (Konig & Klippel, 1963) using a David Kopf No. 900 stereotaxic frame. One animal in each pair received electrical stimulation while the other remained as the sham stimulated control (Collard & Roberts, 1974). In the stimulated animals, stimulus intensity was maintained at 100 μA by measuring the voltage drop across a 40 kΩ resistor and regulating the voltage accordingly. Following raphe stimulation or sham treatment, each forebrain was removed by precollicular section and the 5-HT and 5-HIAA concentration measured as previously described (Collard & Roberts, 1974).

The histological location of electrode placements was identified in all animals receiving stimulation. Whenever an electrode was observed to be outside the nucleus raphe, that result and its sham stimulated pair were rejected.

Measurement of Li^+ in brain and plasma

The concentration of Li^+ in brain and plasma following treatment with Li^+ for 10 days (0.75 mmol/kg per day) was measured in a group of 8 animals. The animals were killed by cervical dislocation and decapitation 24 h after the final injection of Li^+. Blood samples were collected from the neck into heparinised tubes, and each forebrain was removed by precollicular section. Li^+ was extracted from brain and plasma as described by Schou (1958) and measured by flame emission spectrophotometry with an EEL 240 Mark II Atomic absorption spectrophotometer.

Preparation of synaptosomes and measurement of 5-hydroxytryptamine uptake

Synaptosomes were prepared from whole forebrain essentially by the method of Kurokawa, Sakamoto & Kato (1965). In this study however, a greater yield of synaptosomes was obtained by using 2.5% Ficoll instead of 3.0% Ficoll as the middle band in the density gradient. For this reason 2.5% Ficoll was used in all studies.

Two uptake studies were conducted. In the first study, animals were pretreated with Li^+ and chlorimipramine, and in the second study, Li^+ and chlorimipramine were added to synaptosomal suspensions *in vitro*.

5-Hydroxytryptamine uptake by synaptosomes from drug pretreated animals. Two groups of 18 animals were used in the study. Animals were housed in pairs and given free access to food (Rodent breeding diet, Spratts Patent Ltd.) and water. Each animal in 1 group received an injection of LiCl (0.75 mmol/kg i.p.) each day for 10 days while those in the other group received saline; 24 h after the final injection of Li^+ or saline, half of the animals in each group received chlorimipramine (5 mg/kg i.p.) while the others received an equivalent volume of saline. Three and a half hours after the injection of chlorimipramine or saline (corresponding to the time at which raphe stimulation was applied), animals were killed, the forebrain removed and synaptosomes prepared from each animal as described.

Synaptosomes were suspended in Krebs bicarbonate medium containing 1.14×10^{-3} M L-ascorbic acid, 1×10^{-2} M glucose and 1×10^{-4} M iproniazid phosphate. The suspension was gassed with 95% O_2 and 5% CO_2. Aliquots (6.0 ml) of the synaptosomal suspension (protein concentration approx. 1.0 mg) were pre-incubated for 10 min at 37°C. [^{14}C]-5-HT (specific activity 27 mCi/mmol) was then added to give a final concentration of 1×10^{-7} M and the suspension incubated for a further 10 minutes. Following incubation, the synaptosomes were centrifuged at 9,000 × *g* for 10 minutes. The pellet was washed by resuspension in fresh Krebs and recentrifuged at 9,000 × *g* for a further 10 minutes. The 5-HT was extracted by suspending the pellet in 1.0 ml of 0.4 N perchloric acid and centrifuging at 10,000 × *g* for 10 minutes; 0.5 ml of the supernatant was removed for counting and the pellet was dissolved in NaOH for protein estimation by the method of Lowry, Rosebrough, Farr & Randall (1951).

5-Hydroxytryptamine uptake by synaptosomes treated with Li^+ in vitro. In this experiment, synaptosomes were prepared from 10 untreated animals and Li^+ and chlorimipramine were added to the suspension *in vitro*. The final concentration of chlorimipramine in the synaptosomal suspension was 4×10^{-7} M, a concentration known to inhibit 5-HT uptake by about 50% (Schacht & Heptner, 1974).

The concentration of Li^+ used in the incubation medium was calculated on the basis of the findings of DeFeudis (1972). Analysis of the data presented by DeFeudis demonstrated that when referred to the protein concentration of the various subcellular fractions, the amount of Li^+ present in the P_2 fraction (synaptosomes plus mitochondria) was about 0.21 to 0.29% of that present in whole brain homogenates. Based on the measurements of brain Li^+ conducted in this study, this provides a figure of 0.15 to 0.20 nmol Li^+/mg synaptosomal protein. A 6.0 ml aliquot of synaptosomal suspension contains approximately 1.0 mg protein. Thus the final concentration of Li^+ in the incubation medium was 2.5×10^{-8} M and 3.3×10^{-8} M.

Synaptosomes prepared as described above were incubated with 1×10^{-7} M [^{14}C]-5-HT (specific activity 27 mCi/mmol): (1) in the absence of any drug; (2) in the presence of chlorimipramine; (3) in the pres-

ence of Li^+ alone and (4) in the presence of Li^+ and chlorimipramine. The experimental procedure and the extraction of 5-HT were exactly as described above.

Statistical analyses

Raphe stimulation study. In order to remove day to day and within day variations in 5-hydroxyindole concentrations (Quay, 1968; Okada, 1971; Hery, Rouer & Glowinski, 1972) animals were closely paired on a time basis throughout the experiments. The differences between the various treatments were analysed by means of a paired *t* test on time matched pairs.

Uptake studies. The effect of drugs on 5-HT uptake was analysed by Student's *t* test.

Drugs

Lithium chloride (BDH) was administered as an isotonic (0.15 M) solution. Chlorimipramine hydrochloride (Ciba-Geigy) was prepared as a 5 mg/ml solution in saline.

5-Hydroxy [side chain -2-^{14}C] tryptamine creatinine sulphate was obtained from the Radiochemical Centre, Amersham.

Results

Stimulation study

The effect of Li^+ on the concentration of 5-HT and 5-HIAA in unstimulated animals is shown in Table 1. It can be seen that Li^+ had no effect on 5-HT but significantly increased the forebrain concentration of 5-HIAA.

Table 1 also shows the effect of chlorimipramine on the concentration of 5-HT and 5-HIAA in unstimulated animals in both the control and Li^+-treated groups. Chlorimipramine had no significant effect on the forebrain concentration of 5-HT in either group but significantly reduced the 5-HIAA concentration in both groups.

The changes in forebrain 5-hydroxyindoles induced by raphe stimulation in controls and Li^+-treated ani-

Table 1 The effect of Li^+ (0.75 mmol/kg i.p. per day for 10 days) and of chlorimipramine (5 mg/kg i.p.) on the forebrain concentration of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) (ng/g wet weight)

	5-HT concentration (ng/g)		*5-HIAA concentration (ng/g)*	
	Control	*Lithium*	*Control*	*Lithium*
Saline	614 ± 67	562 ± 42	334 ± 17	374 ± 13*
Chlorimipramine	537 ± 56	572 ± 38	294 ± 12	279 ± 10
P (saline vs chlorimipramine)	NS	NS	< 0.05	< 0.001

Results are expressed as the mean ± s.e. means of results from 9 animals and analysed by the paired *t* test. NS: not significant. Asterisk indicates significant difference between Li^+ and control animals, $P < 0.01$

Table 2 The effect of chlorimipramine (5 mg/kg i.p.) on the changes in forebrain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentration induced by 30 min raphe stimulation applied 3.5 h after the injection of chlorimipramine or saline in control animals (10 day saline treatment) and animals which had received 10 day Li^+ treatment.

	Change in 5-HT concentration (ng/g)		*Change in 5-HIAA concentration (ng/g)*	
	Control	*Lithium*	*Control*	*Lithium*
Saline	+44 ± 53	+71 ± 50	+93 ± 12**	+98 ± 21*
Chlorimipramine	+116 ± 59	−42 ± 51	−16 ± 15	+78 ± 14**
P (saline vs. chlorimipramine)	NS	< 0.001	< 0.05	NS

The results are expressed as the mean change ± s.e. mean of results from 9 pairs of animals in each group and analysed by the paired *t* test. (+ and − indicate increase and decreases respectively in 5-hydroxyindole concentrations following raphe stimulation.) NS: not significant. Asterisks indicate significant changes between stimulated and sham-stimulated animals: $*P < 0.01$; $**P < 0.001$.

mals is shown in Table 2. Raphe stimulation increased slightly but not significantly the concentration of 5-HT and increased significantly the concentration of 5-HIAA in both the control ($P < 0.001$) and Li^+ ($P < 0.01$)-treated groups. Chlorimipramine had no effect on the change in 5-HT induced by raphe stimulation in the control group but significantly reduced that seen in the Li^+ group. The increase in 5-HIAA induced by raphe stimulation was completely abolished by chlorimipramine in the control group whereas in the Li^+-treated group chlorimipramine failed to reduce significantly the response to stimulation.

The concentrations of Li^+ in brain and plasma were measured at approximately the same time as the stimulation experiments were conducted; these were, plasma Li^+, 32 ± 2.7 μmol/l and brain Li^+, 70 ± 0.5 μmol/kg ($n = 8$).

Uptake studies

The effect of treatment with chlorimipramine on the uptake of 5-HT in synaptosomes prepared from rats treated for 10 days with saline or Li^+ is shown in Table 3. It can be seen that treatment with chlorimipramine significantly reduced the uptake of 5-HT in both the control (saline-treated) and Li^+-treated groups. Furthermore, the degree of inhibition was similar in both groups.

The result of the study in which the effect of chlorimipramine *in vitro* on the uptake of 5-HT was examined in the presence or absence of Li^+ is shown in Table 4. The uptake of 5-HT was significantly reduced by chlorimipramine in both the presence and absence of Li^+. In addition, the degree to which chlorimipramine reduced the uptake of 5-HT was similar whether Li^+ was present or absent from the incubation medium.

Discussion

Neither Li^+ nor chlorimipramine had any effect on the concentration of 5-HT in the forebrain of animals which had not received raphe stimulation. However, the combined administration of Li^+ and chlorimipramine reduced the forebrain concentration of 5-HT in stimulated animals. This may indicate that the increase in 5-HT synthesis observed following raphe stimulation (Sheard & Aghajanian, 1968) may be prevented by treatment with Li^+ and chlorimipramine.

The concentration of 5-HIAA in the forebrain of unstimulated animals was affected by both Li^+ and chlorimipramine treatment. Li^+ increased the 5-HIAA concentration and chlorimipramine reduced it in both Li^+-treated and saline-treated animals. An increase in 5-HIAA concentration following acute Li^+ treatment has been previously observed and inter-

Table 3 The effect of chlorimipramine (5 mg/kg i.p.) on the uptake of 5-hydroxytryptamine (5-HT) by synaptosomes prepared from control rats and rats treated with Li^+ for 10 days

	5-HT uptake (pmol/mg protein)	
	Saline	*Chlorimipramine*
Control	15.057 ± 0.624	12.699 ± 0.703*
Lithium	14.369 ± 0.549	11.631 ± 0.724**

The results are expressed as the mean ± s.e. mean of results from 9 rats. The effect of chlorimipramine was analysed by the Student's *t* test: *$P < 0.05$; **$P < 0.001$.

Table 4 The effect of chlorimipramine *in vitro* (4×10^{-7} M) on the synaptosomal uptake of 5-hydroxytryptamine (5-HT) in the presence or absence of Li^+ in the incubation medium

	5-HT uptake (pmol/mg protein)	
	No chlorimipramine	*Chlorimipramine*
Control	15.091 ± 1.237	6.801 ± 0.481*
Lithium (2.5×10^{-8} M)	15.228 ± 1.358	6.288 ± 0.469*
Control	11.939 ± 1.455	5.227 ± 0.514*
Lithium (3.3×10^{-8} M)	13.006 ± 1.126	5.633 ± 0.789*

Results are expressed as the mean ± s.e. mean of results from 10 animals. The effect of chlorimipramine was analysed by the Student's *t* test: *$P < 0.001$.

preted as being due to an increase in the synthesis and turnover of 5-HT (Knapp & Mandell, 1973; Poitou, Guerinot & Bohoun, 1974). Treatment with Li^+ for 10 days or more appears to have little effect on turnover (Bliss & Ailion, 1970; Knapp & Mandell, 1973), and it has been suggested that the increase in 5-HIAA following 10 day Li^+ treatment may result in part from an impairment of the storage mechanism within 5-HT terminals (Collard & Roberts, 1977).

The reduction in 5-HIAA concentration following chlorimipramine treatment has been interpreted as being due to the inhibition of 5-HT uptake denying extraneuronally released 5-HT access to intracellular monoamine oxidase rather than to a change in 5-HT synthesis and turnover (Collard & Roberts, 1974). Increasing the activity in 5-HT neurones by stimulation of the nucleus raphe medianus should therefore enhance the effect of chlorimipramine on the concentration of 5-HIAA (Samanin *et al.*, 1972; Collard & Roberts, 1974). This was the case in the control group of animals where the increase in 5-HIAA concentration by raphe stimulation was completely abolished by chlorimipramine. However, in the Li^+-treated group the increase in 5-HIAA following raphe stimulation was unaffected by chlorimipramine. The difference observed between the 2 groups may indicate that in the Li^+-treated group the 5-HIAA produced by stimulation is derived from 5-HT which remained predominantly in the intracellular compartment, or that Li^+ is antagonizing the inhibition of 5-HT uptake by chlorimipramine. If the latter were the case, released 5-HT would still be able to gain access to intracellular monoamine oxidase.

In order to investigate the second possibility, the interaction between Li^+ and chlorimipramine on synaptosomal uptake of 5-HT was examined. Both treatment with Li^+ and the addition of Li^+ to synaptosomal suspensions had no apparent effect on the ability of chlorimipramine to inhibit 5-HT uptake into synaptosomes. The small extent to which uptake was inhibited by prior treatment with chlorimipramine was probably due to loss of the drug during the extraction of the synaptosomes. The low-affinity binding of the related tricyclic antidepressant, imipramine, to synaptosomal membranes has been shown to dissociate during subcellular fractionation (Hunt, Kannengiesser & Raynauld, 1975). Although it is not known whether the binding investigated by Hunt *et al.* (1975) is related to the role of imipramine as an inhibitor of 5-HT uptake, it is possible that loss of the drug during isolation of synaptosomes could have influenced the result in the present study.

It would appear therefore that the increase in 5-HIAA produced by raphe stimulation in the Li^+-treated animals may well be derived from the metabolism of 5-HT which remains within the intracellular compartment. Within the nerve terminal it is believed that stored 5-HT is protected from deamination while free cytoplasmic 5-HT can be metabolized by monoamine oxidase (Moir & Eccleston, 1968). This it can be suggested that Li^+ interferes with stimulus-release coupling in such a way that 5-HT is released from the storage sites into the cytoplasm rather than into the synaptic cleft. Such an effect would require Li^+ to interfere with the location of the transmitter store within the nerve terminal membrane.

If Li^+ is interfering with stimulus-release coupling in the manner described above, inhibition of monoamine oxidase in Li^+-treated animals should reduce the intracellular deamination of 5-HT and increase the leakage of 5-HT from the presynaptic terminal. Evidence that this occurs is provided by Grahame-Smith & Green (1974) who observed that the administration of tranylcypromine or pargyline to Li^+ pretreated animals increased the amount of 5-HT reaching receptors.

A further point of interest from this present study is the fact that chlorimipramine was able to reduce the resting concentration of 5-HIAA in Li^+-treated animals, but not that produced by stimulation. This may indicate that the proposed effect of Li^+ on 5-HT release is only manifest during periods in which the 5-HT neurones are highly active.

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