of the effects of imipramine on behavior may involve a change in the level of 5-HT at receptor site in the brain. A further investigation is now underway to determine the effects of 5-hydroxytryptophan, a 5-HT precursor, and related catecholamines on these behavioral measures.

REFERENCES

- 1) KAMEYAMA, T., SHIGEHISA, T.: *Japan. J. Pharmacol.*, **23**, Suppl. 62 (1973); 2) KAMEYAMA, T., SHIGEHISA, T.: *Japan. J. Pharmacol.*, **23**, Suppl. 63 (1973); 3) CARLSSON, A., CORRODI, H., FUXE, K., HÖKFELT, T.: *Europ. J. Pharmacol.*, **5**, 357 (1969); 4) CARLSSON, A.: *J. Pharm. Pharmacol.*, **22**, 729 (1970); 5) SHASKAN, E.G., SNYDER, S.H.: *J. Pharmacol. exp. Ther.*, **175**, 404 (1970); 6) SHEARD, M.H., ZOLOVICK, A., AGHAJANIAN, G.K.: *Brain Res.*, **43**, 690 (1972); 7) SNYDER, S.H., AXELROD, J., ZWIG, M.: *Biochem. Pharmacol.*, **14**, 831 (1965)

PRE- AND POST-SYNAPTIC ORIGIN OF LITHIUM-INDUCED ACUTE BLOCKADE ON ADRENERGIC TRANSMISSION IN RABBIT HEART

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Accepted September 18, 1975

Lithium is being clinically prescribed for patients in the manic phase of manic depressive psychosis. There are reports that lithium interferes with metabolism (1-3) and release of noradrenaline (4) in the brain and that iontophoretically applied lithium antagonises a noradrenaline-induced inhibition of hippocampal spontaneous firing activity in rats (5). The present experiment was carried out to investigate the action of lithium on the adrenergic transmission in perfused rabbit hearts with sympathetic trunks as a model. Pre- and post-synaptic responses to nerve stimulation could thus be obtained simultaneously.

The hearts with bilateral sympathetic trunks from 33 rabbits of either sex, 1.6 to 2.0 kg, were isolated and perfused with Krebs bicarbonate solution gassed with 5% CO₂ in oxygen as described previously (6). Temperature of the solution was kept at 30°C. The perfusion pressure was 60 cm H₂O and the rate was 10 to 15 ml/min. The extreme upper thoracic region of nerves was stimulated through ring electrodes similar to those described by Garry and Gillespie (7) with supramaximal rectangular pulses of 3.5 msec duration at a frequency of 30 Hz. The nerves were stimulated 6 times, each of 30 sec duration with a 30 sec resting period. This 6 min stimulation-rest procedure is referred to as the 1st procedure. The 2nd procedure was performed similarly 30 min after the beginning of the 1st procedure. The venous effluent was collected for each 6 min procedure period and noradrenaline was measured fluorometrically by the methods of Anton and Sayre (8).

Some hearts were suspended by means of a perfusion apparatus KN-206 (Natsume)

for recording contractile responses to exogenously applied noradrenaline or isoproterenol. Responses were recorded on smoked paper with a semi-isotonic lever. Student's *t*-test was used to evaluate data.

In untreated hearts, the amount of noradrenaline released during the 1st procedure varied from 93 to 711 ng. The mean was 311.8 ± 93.9 ng. During the 2nd procedure, as shown in Fig. 1-A (right ordinate), the output of noradrenaline was relatively constant 29.9 $\pm$ 4.0% of that during the 1st procedure, the value taken as control. There were marked increases in the heart rate during the both procedures. The initial heart rate was 100 ± 4 /min. The percent increase in chronotropic response to each 30 sec stimulation of the 1st procedure was 64.2 ± 6.0 , 64.7 ± 4.3 , 61.3 ± 3.5 , 55.6 ± 5.0 , 54.6 ± 4.9 and 55.8 ± 7.2 . The heart rate returned to normal within a few min after the 1st procedure was over and was 91 ± 3 before the 2nd procedure. Chronotropic responses to the 2nd procedure were much the same as those of the 1st procedure and are shown in circles of Fig. 1-A as a % of the corresponding response to the 1st procedure (left ordinate). The coronary flow decreased by 5 to 10% during the both procedures. These results are consistent with those in a previous report (6).

After the 1st procedure, hearts were perfused with a test solution prepared by an addition of LiCl 14.3 mM in Fig. 1-C. The heart rate after the solution was changed and the coronary flow during both procedures was the same as observed in the untreated hearts. The output of noradrenaline decreased to $18.2 \pm 1.9\%$ of that before the perfusion, the value being significant ($P < 0.01$) from control. Each chronotropic response decreased progressively, the

FIG. 1. Effects of lithium on adrenergic transmission in isolated rabbit hearts.

Sympathetic nerve was stimulated 6 times (the 1st procedure), each of 30 sec duration with 30 sec resting period, during perfusion with untreated Krebs solution. Thereafter, hearts were perfused with a test solution prepared by an addition of LiCl or sucrose (B to D). In a similar manner the 2nd procedure was started 24 min after changing the solution. Abscissa shows the number of each 30 sec stimulation of both procedures. Heart beats were counted from 15 to 45 sec after the start of each 30 sec stimulation. The output of noradrenaline released (column) and each positive chronotropic response (circle) during the 2nd procedure are demonstrated as a % of that during the 1st procedure, respectively. The number of experiments is shown in parentheses. Vertical bars indicate standard errors.

3rd to 6th response being different ($P<0.01$) from the corresponding response in untreated hearts, respectively. Decrease in the output of noradrenaline involved in the transmission blockade cannot be attributed to hypertonicity of the lithium solution, as there was no decrease in noradrenaline output following perfusion with an equi-osmotic medium containing sucrose (Fig. 1-D). Katz and Kopin (4) have shown that in brain slices of rats a lithium-induced inhibition of ^3H -noradrenaline release evoked by electrical stimuli is prevented by raising the concentration of calcium. The site of the lithium action in rabbit hearts appears to be mainly in adrenergic nerve endings.

As shown in Fig. 1-B, the 4th to 6th positive chronotropic response of 7.2 mM lithium perfused hearts decreased significantly ($P<0.05$) from that in untreated hearts, at a time when the output of noradrenaline showed no difference ($25.1\pm4.7\%$) from control. The decrease in chronotropic responses is not derived from the condition of repetitive nerve stimulation, as adrenergic transmission induced by a once only 30 sec stimulation in rabbit atria was significantly inhibited 30 min after the addition of lithium 7.2 mM (9). These results suggest that the major mechanism of the transmission blockade induced by lithium 7.2 mM is of a post-synaptic origin.

In Table 1, peak positive responses were observed within 1 min of noradrenaline injection and 1 to 3 min after isoproterenol injection. Before perfusion with 7.2 mM lithium solution, the duration of noradrenaline actions was 4 to 9 min and that of isoproterenol was 11 to 20 min or more in case of 4.0×10^{-6} M. Peak responses to noradrenaline after lithium perfusion decreased in parallel with the transmission blockade. The duration of the actions also decreased to 3 to 7 min. Chronic treatment with lithium was reported to

TABLE 1. Positive chronotropic and contractile responses of rabbit hearts to exogenously applied noradrenaline or isoproterenol before (control) and 30 min after perfusion with the medium containing LiCl 7.2 mM

	M	No. of hearts	Positive chronotropic responses		Positive contractile responses	
			Control	After LiCl 7.2 mM	Control	After LiCl 7.2 mM
dl-Noradrenaline	2.4×10^{-5}	4	17.1 ± 3.9	9.7 ± 2.4	44.9 ± 8.6	$13.4\pm3.3^{**}$
	4.9×10^{-6}	6	23.1 ± 2.8	$13.2\pm2.7^*$	57.8 ± 6.3	$24.3\pm3.8^{**}$
	2.4×10^{-4}	6	58.9 ± 3.4	$43.6\pm4.1^*$	96.6 ± 6.6	$64.0\pm3.4^{**}$
l-Isoproterenol	1.6×10^{-7}	7	18.0 ± 3.5	$8.9\pm1.6^*$	28.5 ± 4.8	$11.5\pm3.8^*$
	8.1×10^{-7}	7	34.4 ± 4.4	$15.0\pm2.1^{**}$	57.4 ± 7.5	$29.3\pm4.8^{**}$
	4.0×10^{-6}	7	51.3 ± 5.9	$31.5\pm5.0^*$	67.5 ± 3.2	$44.3\pm7.7^*$

The amine, a hydrochloride, was injected in the order from low to high dose into the aortic cannula. Intervals of injection were 6 to 9 min for noradrenaline and 8 to 20 min for isoproterenol. The concentration of injection solution is expressed in terms of M. Injection volume was 0.1 ml. Heart beats were counted for 30 sec, 15 sec and every 1 min after injection. Peak responses are expressed as % increases (mean $\pm$ standard error).

*: Significantly different from control, $P<0.05$.

**: Significantly different from control, $P<0.01$.

produce an increase in the uptake of ^3H -noradrenaline *in vitro* by synaptosomes isolated from the rat brain (10). Lithium may thus be considered to increase noradrenaline uptake by adrenergic endings in the heart, which would in turn result in a decrease in the transmitter available for interaction with receptors. To rule out such a possibility, isoproterenol was used, as the amine is not a substrate for the neuronal uptake mechanism (11). Peak responses to isoproterenol after lithium perfusion also decreased in parallel with the transmission blockade. The duration of the actions decreased to 8 to 16 min. The results are consistent with the findings that positive inotropism as well as the activation of phosphorylase A by noradrenaline and isoproterenol decreased after perfusion with a 10 mM lithium solution in guinea-pig hearts (12).

Thus it is concluded that the primary blocking action of lithium on the adrenergic transmission is not due to a decrease in the noradrenaline which interacts with receptors but rather to an inhibition of post-synaptic responses of the rabbit heart to the transmitter.

REFERENCES

- 1) CORRODI, H., FUXE, K., HÖKFELT, T. AND SCHOU, M.: *Psychopharmacologia* **11**, 345 (1967);
- 2) SCHANBERG, S.M., SCHILDKRAUT, J.J. AND KOPIN, I.J.: *Biochem. Pharmacol.* **16**, 393 (1967);
- 3) SCHILDKRAUT, J.J., LOGUE, M.A. AND DODGE, G.A.: *Psychopharmacologia* **14**, 135 (1969);
- 4) KATZ, R.I. AND KOPIN, I.J.: *Biochem. Pharmacol.* **18**, 1935 (1969); 5) SEGAL, M.: *Nature* **250**, 72 (1974); 6) MISU, Y., AND KUBO, T.: *Japan. J. Pharmacol.* **22**, 359 (1972); 7) GARRY, R.C. AND GILLESPIE, J.S.: *J. Physiol.* **128**, 557 (1955); 8) ANTON, A.H. AND SAYRE, D.F.: *J. Pharmacol. exp. Ther.* **138**, 360 (1962); 9) MISU, Y., KURAISHI, Y. AND TAKAGI, H.: *Japan. J. Pharmacol.* **23**, 891 (1973); 10) COLBURN, R.W., GOODWIN, F.K., BUNNEY, W.F., JR. AND DAVIS, J.M.: *Nature* **215**, 1395 (1967); 11) IVERSEN, L.L. AND CALLINGHAM, B.A.: *Fundamentals of Biochemical Pharmacology*, Edited by BACQ, Z.M., ČAPEK, R., PAOLETTI, R. AND RENSON, J., p. 253, PERGAMON Press, Oxford, New York, Toronto, Sydney, Braunschweig (1971); 12) FRAZER, A., HANCOCK, A., MENDELS, J. AND MACINTIRE, R.: *Biol. Psychiat.* **5**, 79 (1972)

QUANTITATIVE ASPECT OF ACID SECRETION RESPONSE OF ISOLATED BULLFROG GASTRIC MUCOSA PREPARATION: EFFECT OF SECRETAGOGUES AND INHIBITORS

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Accepted October 1, 1975

In recent years, a considerable number of studies concerning the physiological functions of gastrointestinal hormones have been done (1-3). The gastric acid secretion is, however, subjected to complex neural and hormonal influences. Therefore, the simple experimental system using an isolated organ is necessary in order to elucidate the mechanisms of actions of the secretagogues on gastric acid secretion. The bullfrog (*Rana catesbiana*) gastric mucosa can be readily separated from the muscular layers without giving any injury to the