Effects of ATE administration on plasma glucose and hepatic glycogen levels of intact and adrenalectomized rats

Treatment	Time (h)	Intact animals[a]		Adrenalectomized animals[b]	
		Plasma glucose[c]	Hepatic glycogen[d]	Plasma glucose[c]	Hepatic glycogen[d]
Control	1	139 ± 3[e]	48 ± 2	126 ± 3	24 ± 3
ATE	1	185 ± 8[f]	40 ± 3	168 ± 4[f]	14 ± 3[f]
Control	2.5	150 ± 5	32 ± 2	143 ± 4	24 ± 3
ATE	2.5	136 ± 6	22 ± 4[f]	73 ± 12[f]	6 ± 3[f]
Control	5	168 ± 9	26 ± 1	145 ± 4	20 ± 7
ATE	5	44 ± 11[f]	0.1 ± 0.1[f]	32 ± 14[f]	1 ± 0.6[f]

[a] 75 mg/kg. [b] 5 mg/kg. [c] mg/100 ml. [d] mg equivalents glucose/g liver tissue. [e] Mean ± S.E. 6 animals. [f] $p < 0.05$.

Hypoglycemia reflects a disturbance in one of the many physiologic, or enzymatic controls that maintain normoglycemia. We propose that ATE-induced hypoglycemia is the result in part of accelerated mobilization of hepatic glycogen stores, analogous to the hypoglycemia of endotoxicosis[6] and glucagon administration[7].

Résumé. L'administration i.v. d'un extrait aqueux de tissu adipeux (ATE) à des rats intacts surrénalectomisés provoque un état de glycogénolyse hépatique suivi d'hypoglycémie. La glycogénolyse semble être indépendante de l'épinéphrine. L'hypoglycémie provoquée par l'ATE résulte en partie de la mobilisation accélérée des réserves de glycogène hépatique.

B. W. Siegel, T. R. Blohm and N. L. Wiech

Merrell-National Laboratories, Division of Richardson-Merell Inc., Metabolic Diseases Department, Cincinnati (Ohio 45215, USA), 19 August 1974.

[6] J. P. Sanford, J. A. Sanford and C. Gott, J. exp. Med. *112*, 97 (1960).
[7] B. W. Siegel, N. L. Wiech and T. R. Blohm, Fedn. Proc. *32*, 311 (1973).

Effect of Lithium and other Drugs on the Amphetamine Chlordiazepoxide Hyperactivity in Mice

The action of lithium on psychotic excitement has been shown by Cade[1] as early as 1949. Since then, its efficacity in the prevention of the manic-depressive psychosis has been well established[2,3]. Nevertheless, there are few observations on the effects of lithium on the behaviour of laboratory animals. Indeed, the action of lithium is very peculiar and cannot be compared with any reference drug; indeed, many authors think lithium acts specifically on the manic-depressive disease and cannot act out of its clinical context[4]. Nevertheless, it would be very surprizing if such a product had no effect on animals. Weisher[5], Sheard[6], Brain[7] and Eichelman et al.[8] have noted a decrease of the aggressivity in different animal species. Johnson and Wormington[9] have found that the lithium treatment decreased the rearing activity of the rat, without modifying its horizontal activity[10]. Perkinson et al.[11] have found an inhibition of the tetrabenazine depression. Moreover, some authors have studied the action of lithium on experimental agitation states, which were even called 'manic'. Matussek and Linsmayer[12] have shown that lithium decreased the rat hyperactivity, produced by the administration of desmethyl-imipramine and a reserpine-like benzoquinolizine (Ro 4.1284), but has no effect on the amphetaminic excitement. Caroll and Sharp[13] have observed that the lithium salts decreased the morphine hyperactivity in mice. Finally, lithium has been shown to decrease the hyperactivity of rats and mice, produced by the chlordiazepoxide-amphetamine association[14,15].

During this study, we attempted to reproduce this type of induced hyperactivity, and, after checking the effect of lithium, we studied other drugs under the same conditions.

Material and methods. The study was made with female Swiss mice (20–25 g), kept in cages of 10 animals. Treatment was the association of dexamphetamine bitartrate (5 mg/kg) and chlordiazeproxide (25 mg/kg), in solution in NaCl 0.9%. The volume injected was 0.20 ml per 20 g of body weight, the injections were made i.p. The behavioural effect was studied with a holeboard[16] 20 min after the injection. These doses and latency times were those of U'Prichard and Steinberg[15]. The control mice received the same volume of NaCl 0.9%.

[1] J. F. J. Cade, Med. J. Aust. *36*, 349 (1949).
[2] M. Schou, Encéphale *60*, 281 (1971).
[3] M. Schou and D. M. Shaw, Practitionner *210*, 105 (1973).
[4] M. Schou, Biochem. Soc. Transact. *1*, 81 (1973).
[5] M. L. Weisher, Psychopharmacologia *15*, 245 (1969).
[6] M. H. Sheard, Nature, Lond. *228*, 284 (1970).
[7] P. F. Brain, J. Endocr. *55*, 39 (1972).
[8] B. Eichelman, N. B. Thoa and J. Perez-Cruet, Pharmac. Biochem. Behav. *1*, 121 (1973).
[9] F. N. Johnson and S. Wormington, Nature New Biol. *235*, 159 (1972).
[10] F. N. Johnson, Experientia *28*, 533 (1972).
[11] E. Perkinson, R. Ruckart and J. P. da Vanzo, Proc. Soc. exp. Biol. Med. *131*, 685 (1969).
[12] N. Matussek and M. Linsmayer, Life Sci. *7*, 371 (1968).
[13] B. J. Caroll and P. T. Sharp, Science *172*, 1355 (1971).
[14] C. Cox, P. E. Harrison-Read, H. Steinberg and H. Tomkiewicz, Nature, Lond. *232*, 336 (1971).
[15] D. C. U'Prichard and H. Steinberg, Br. J. Pharmac. *44*, 349 (1972).
[16] J. R. Boissier and P. Simon, Archs int. Pharmacodyn., Thér. *147*, 372 (1964).

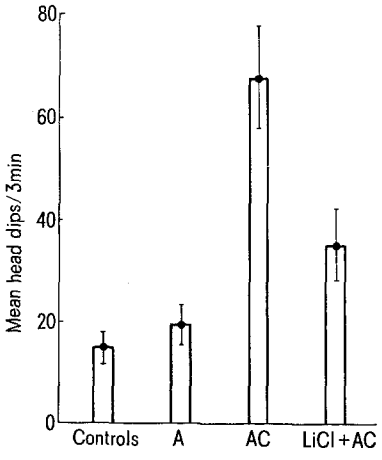

Fig. 1. Effect of lithium on the mixture-induced hyperactivity. A, amphetamine bitartrate (5 mg/kg); AC, amphetamine bitartrate (5 mg/kg) + chlordiazepoxide (25 mg/kg).

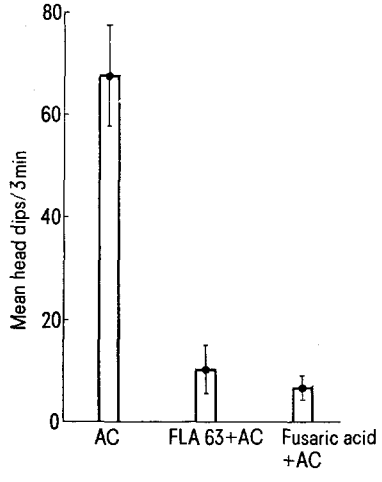

Fig. 2. Effect of dopamine B hydroxylase inhibitors in the mixture-induced hyperactivity.

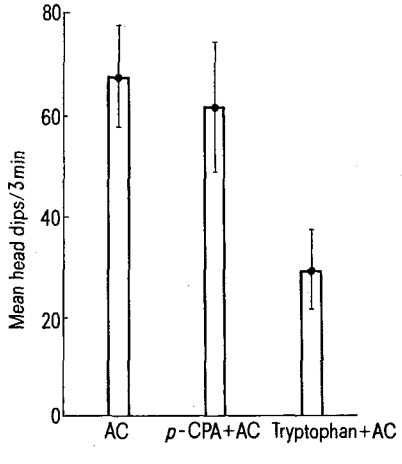

Fig. 3. Effect of tryptophan and *p*-chlorophenylalanine on the mixture-induced hyperactivity.

The lithium-treated group received 0.39 ml for 20 g of body weight of isotonic lithium chloride (3 mEq/kg) 3 h before the association. The other treatments, except *p*-chlorophenylalanine, were made 1 h before the association, and were dissolved in NaCl 0.9% at a suitable pH. The results expressed the number of head dips of the mouse within 3 min. The head dips were scored only if both eyes disappeared into the hole.

Results. Under the conditions described, the dexamphetamine-chlordiazepoxide association induced a peculiar excitement state in mice: the mice made high scores but only explores a few holes; they successively dipped their heads into the same hole. The previous administration of lithium (3 mEq/kg i.p.), 3 h before, produced a significant decrease of the score (Figure 1). Although it is not possible to demonstrate a possible anti-amphetamine effect of lithium on this test, it does not seem to be connected: MATUSSEK and LINSMAYER[12], and FRAISSE[17] have not found a lithium effect on the amphetaminic hyperactivity.

The previous treatment with 2 types of dopamine-β-hydroxylase inhibitors, FLA 63 (50 mg/kg) and fusaric acid (75 mg/kg) (Figure 2) has shown the same inhibitory effect on the 'manic test'.

The serotonin precursors, L-tryptophan (300 mg/kg) and L-5-hydroxytryptophan (100 mg/kg) have been shown to inhibit this phenomenon, and so to act in the same way as lithium. It has been shown that L-tryptophan, at this dose, increased the serotonin synthesis in the brain[18]. However, *p*-chlorophenylalanine, which depletes serotonin (300 mg/kg, 48 h before), has no effect on this test.

Discussion. The effect of lithium on this experimental model of agitation is in agreement with the results of authors[15] who used this model and with those of other workers using different experimental situations and methods of inducing hyperactivity[12,13]. The action of inhibitors of dopamine-β-hydroxylase shows that an important release of norepinephrine is probably involved in this experimental hyperactivity, as in MATUSSEK and LINSMAYER's[12]. Moreover, STEINBERG[19] and DAVIES et al.[20] had shown that α-methyl-*p*-tyrosine, which depleted catecholamines by tyrosine hydroxylase inhibition, antagonized the hyperactivity produced by this association. It may be thought that lithium acts either by decreasing the sensibility of norepinephrine receptor, or by decreasing the mediator liberation. This second hypothesis agrees with the results of SCHILDKRAUT et al.[21], who have noted an increase of the intraneuronal catabolism of norepinephrine, and those of KATZ et al.[22] who have shown that lithium decreased the evoked liberation of norepinephrine by brain slices.

The similar effect of lithium, tryptophan and 5-HTP quite agrees with the increased turn-over of brain serotonin, observed by some authors[23–25] in lithium-treated

[17] B. FRAISSE, Thèse Médecine (Paris 1970).
[18] A. CARLSSON and M. LINDQVIST, J. Neur. Transm. *33*, 23 (1972).
[19] H. STEINBERG, Biochem. Soc. Transac. *1*, 93 (1973).
[20] C. DAVIES, D. J. SANGER, H. STEINBERG, M. TOMKIEWICZ and D. C. U'PRICHARD, Psychopharmacologia *36*, 263 (1974).
[21] J. J. SCHILDKRAUT, M. A. LOGUE and G. A. DODGE, Psychopharmacologia *14*, 135 (1969).
[22] R. I. KATZ, T. N. CHASE and I. J. KOPIN, Science *162*, 466 (1968).
[23] J. PEREZ-CRUET, A. TAGLIAMONTE, P. TAGLIAMONTE, G. L. GESSA, J. Pharmac. exp. Ther. *178*, 325 (1971).
[24] J. SCHUBERT, Psychopharmacologia *32*, 301 (1973).
[25] P. POITOU, F. GUERINOT and C. BOHUON, Psychopharmacologia *38*, 75 (1974).

rodents. More, PRANGE et al.[26] have observed an efficacity of tryptophan in mania, especially in the hyperactivity. SHEARD[27] has shown that a lithium inhibition of the *p*-chlorophenylalanine induced aggressivity. Lithium may act by increasing serotonin synthesis, which quite agrees with the increased turn-over of brain serotonin observed by some authors[23–25]. However, this hypothesis is not in agreement with the antagonistic effect of lithium on 5-HTP induced head-twitches in mice, observed by KISELEVA and LAPIN[28]. Thus the simultaneous administration of dexamphetamine and chlordiazepoxide may disturb a norepinephrine-serotonin balance, and it might be prevented by lithium. This effect might explain the action of lithium in mania.

Résumé. L'administration simultanée de dexamphétamine et de chlordiazepoxide provoque, chez la souris, un syndrôme d'hyperactivité, objectivé à l'aide d'une planche à trous. Ce phénomène est inhibé par l'administration préalable de lithium, mais aussi par des inhibiteurs de dopamine-β-hydroxylase et par les précurseurs de sérotonine. Ces résultats suggèrent que le lithium pourrait agir sur une balance noradrénaline sérotonine qui serait perturbée dans la manie.

P. POITOU, R. BOULU and C. BOHUON

Laboratoire de Biologie Clinique et Expérimentale, Institut Gustave-Roussy, 16 bis, Avenue Vaillant-Couturier, F–94800 Villejuif (France); and Laboratoire de Pharmacodynamie, Faculté des Sciences Pharmaceutiques et Biologiques, 4, Avenue de l'Observatoire, F–75006 Paris (France), 26 April 1974.

[26] A. J. PRANGE, I. C. WILSON, C. W. LYNN, L. B. ALLTOP and R. A. STIKELEATHER, Arch. gen. Psychiat. *30*, 56 (1974).
[27] M. H. SHEARD, Commun. Behav. Biol. *5*, 71 (1970).
[28] I. P. KISELEVA and L. P. LAPIN, Pharmac. Res. Commun. *1*, 108 (1969).

Influence of Lithium Chloride on Adrenergic Mechanisms in Ventricle of Frog Heart and Guinea-Pig Left Atrium

The catecholamine uptake system by sympathetic nerve endings of the heart is a process requiring sodium in the incubation medium[1–6]. However, lithium can replace sodium ions in the mechanism generating the cardiac action potential[7]. Splenic slices were able to accumulate H^3-norepinephrine (H^3NE) (60% of their controls) after their depletion of adrenergic transmitter by sodium deprivation in the incubation medium[8]. These facts prompted us to study if the NE uptake system by isolated frog ventricle can be modified by the replacement of sodium by lithium chloride in the incubation medium. On the other hand, the replacement of sodium by lithium in the external medium would depress the sodium-potasium pump activity as sodium concentration into the cell is reduced[9]. The facts lead us to think that the presence of lithium chloride (LiCl) in the incubation medium would prevent the NE incorporation by nerve endings.

In the present paper we have elucidated whether the prevented NE uptake by lithium is or is not produced by a cocaine-like effect by which a supersensitivity to the NE could be expected.

Methods. Experiments with frog ventricle. Ventricles (*Rana pipiens*) were prepared and mounted as previously described by FURCHGOTT et al.[10] for isolated atrium of guinea-pig. Halves were suspended in an organ bath containing 20 ml of regular Ringer solution of the following composition (expressed in m*M*): NaCl, 103.4; KCl, 1.013; $CaCl_2$, 0.9009; CO_3HNa, 1.851, containing 10^{-5} g/ml of ethylene diaminetetraacetic acid (EDTA). A mixture of 95% O_2 and 5% CO_2 was bubbled through the bathing solution. All preparations were electrically driven at a frequency of 30 beats/min. Ventricles were attached to a force-displacement transducer Grass model FT03, and mechanical activity was recorded by means of a Grass Polygraph. Each ventricle was subjected to a resting tension of 1 g. Under their respective conditions, halves were then incubated with 5 ng/ml of D,l-H^3NE for 5 min and then thoroughly washed. 4 additional washes were given over the subsequent 40 min period, at the end of which the halves were removed for analysis of radioactivity. All preparations were performed at room temperature. The catecholamine extraction was performed according to the method of ANTON and SAYRE[11] and radioactivity was counted in a Nuclear Chicago Liquid Scintillation Spectrometer model 725. All samples were corrected for quenching with an automatic external reference standard. Under our working conditions, the radioactivity present in the alumina eluates cannot be ascribed to metabolites of H^3NE but to H^3NE itself[12]. H^3NE is expressed in terms of disintegrations per min/g of tissue (dpm/g). When we refer to H^3NE uptake, we mean H^3NE uptake and retention by isolated ventricle of frog heart. Statistical significance of the difference between means was determined by the *t*-test for paired data. In the experiments with lithium chloride (LiCl), sodium chloride of the Ringer solution was replaced by equimolar amounts of LiCl.

Experiments with isolated atrium of guinea-pig. Atria were mounted in a similar manner as that described above, but suspended in Krebs bicarbonate solution. In the experimental preparations, 100 m*M*/l of LiCl was added to the normal Krebs whereas control preparations received equimolar amounts of NaCl. All preparations were performed at 37 °C. The drugs used were: D-l-norepinephrine-7-H^3-hydrochloride, specific activity 16.7 Ci/m*M* (New England Nuclear Corp.); norepinephrine

[1] W. D. HORST, I. J. KOPIN and E. R. RAME, Am. J. Physiol. *215*, 4 (1968).
[2] L. L. IVERSEN and E. A. KRAVITZ, Molec. Pharmac. *2*, 360 (1966).
[3] D. F. BOGDANSKI and B. B. BRODIE, Life Sci. *5*, 1563 (1966).
[4] C. N. GILLIS and D. M. PATON, Br. J. Pharmac. *29*, 309 (1967).
[5] D. M. PATON, Comp. Biochem. Physiol. *28*, 477 (1969).
[6] P. M. KEEN and D. F. BOGDANSKY, Am. J. Physiol. *219*, 677 (1970).
[7] E. E. CARMELIET, J. gen. Physiol. *47*, 501 (1957).
[8] A. G. GARCIA and S. M. KIRPEKAR, Br. J. Pharmac. *47*, 729 (1973).
[9] J. C. SKOU, Biochim. biophys. Acta *23*, 394 (1957).
[10] R. F. FURCHGOTT, S. M. KIRPEKAR, M. RIEKER and A. SCHWAB, J. Pharmac. exp. Ther. *142*, 39 (1963).
[11] A. H. ANTON and D. F. SAYRE, J. Pharmac. exp. Ther. *138*, 360 (1962).
[12] A. R. WAKADE and R. F. FURCHGOTT, J. Pharmac. exp. Ther. *163*, 123 (1968).