Evoked Release of Norepinephrine and Serotonin from Brain Slices: Inhibition by Lithium

Abstract. *Slices of mammalian brain accumulate H^3-norepinephrine and H^3-serotonin when incubated in a physiologic medium containing these tritiated monoamines. When these tissues are subjected to mild electrical stimulation of short duration, which is associated with depolarization of nerve membranes, a striking increase in the rate of efflux of the exogenous labeled monoamines occurs. Stimulation-induced release of both labeled monoamines is diminished by the presence of lithium ions in the perfusing medium; related monovalent cations had no such effect. Evoked release from slices of brain from animals treated intraperitoneally with lithium chloride for 3 days was also reduced.*

Evidence supporting the hypothesis that a disturbance in amine metabolism occurs in affective disorders has been reviewed (*1*). Many drugs useful in the treatment of these disorders produce characteristic alterations in the metabolism of monoamines in man as well as in the experimental animal (*2*). Lithium salts appear to be effective in the treatment of mania and hypomania (*3*) and to influence the metabolism of norepinephrine in vivo (*4, 5*). It is of interest, therefore, to examine the effects of lithium on release of amines from central nervous system tissue.

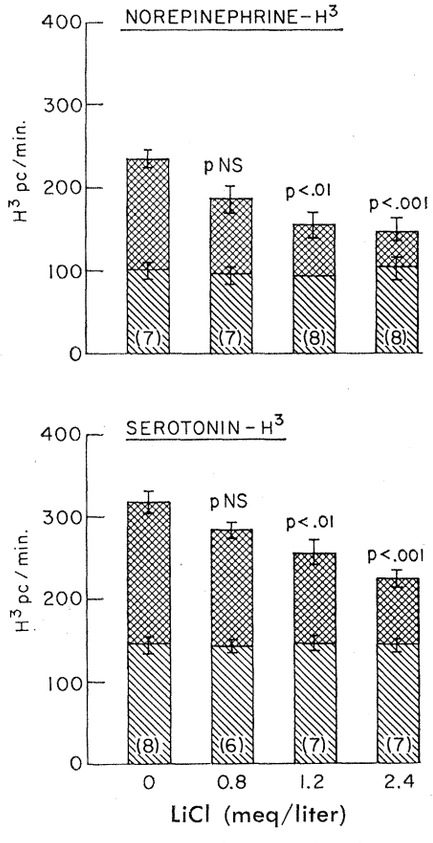

Fig. 1. Effect of lithium ion in vitro on release of monoamine. Rat striatal slices were incubated with tritiated amine, superfused, and stimulated. The lined areas of the bars indicate the tritium efflux before stimulation, and the total height of each bar depicts efflux during stimulation. The cross-hatched areas thus represent stimulation-induced release. Results (pc/min) are the means (± S.E.M.) for the number of slices indicated in parentheses at the foot of each bar. The *P* values refer to differences in the stimulation-induced release of tritium from control slices. Concentrations of lithium ions in the superfusing solutions are given on the abscissa.

Electrical stimulation of mammalian brain slices previously incubated with a labeled monoamine has been used to study the stimulus-induced release of several putative cerebral neurotransmitters (*6, 7*). Tracer amounts of H^3-norepinephrine (*8*) or H^3-serotonin (*9*) are actively accumulated by thin slices of cerebral tissue and appear to be concentrated within nerve terminal structures which normally contain the endogenous amine (*9, 10*). Electrical-field stimulation of a type known to reversibly displace resting membrane potentials (*11*) enhances the efflux of exogenous norepinephrine (*6*) or serotonin (*7*) from brain slices. Having used this technique, we now report a significant diminution in the stimulus-induced release of both norepinephrine and serotonin from rat brain slices by prior lithium treatment of the living animal or upon exposure of isolated cerebral tissue to this ion.

Adult male Sprague-Dawley rats received sodium chloride or lithium chloride, 2.5 meq/kg or 7.5 meq/kg, intraperitoneally, 48 and 24 hours before the experiment. Animals were killed by cervical fracture, their brains were removed rapidly, and 20-mg slices of striatum were prepared (*7*). The slices were incubated at 37°C in a supplemented Krebs-Ringer solution (*8*) saturated with 5 percent carbon dioxide in oxygen and containing *l*-norepinephrine-7-H^3 (8 c/mmole, 25 ng/ml) or H^3-serotonin (2 c/mmole, 50 ng/ml) (New England Nuclear). Uptake of isotopic norepinephrine was identical in tissues from treated and untreated animals.

After 30 minutes, tissues were transferred to individual chambers through which fresh medium was rapidly circulated. When the spontaneous efflux of radioactivity diminished and became nearly steady (about 20 minutes), rectangular, d-c, pulsatile stimulation (9 ma, 100 hz, 4.0 msec) was applied for 1 minute with a Grass S-4 stimulator and CCU-IA constant current control unit. Effluent superfusate was collected during 2-minute intervals immediately before and during stimulation and assayed for total tritium by liquid-scintillation spectroscopy. Stimulation of brain slices which have been incubated with H^3-norepinephrine or H^3-serotonin produce a rapid rise in the tritium efflux, consisting mainly of the unmetabolized amine.

Addition of lithium chloride (0.8, 1.2, or 2.4 meq/liter) to the perfusion medium produced no alteration in the rate of spontaneous efflux of the labeled

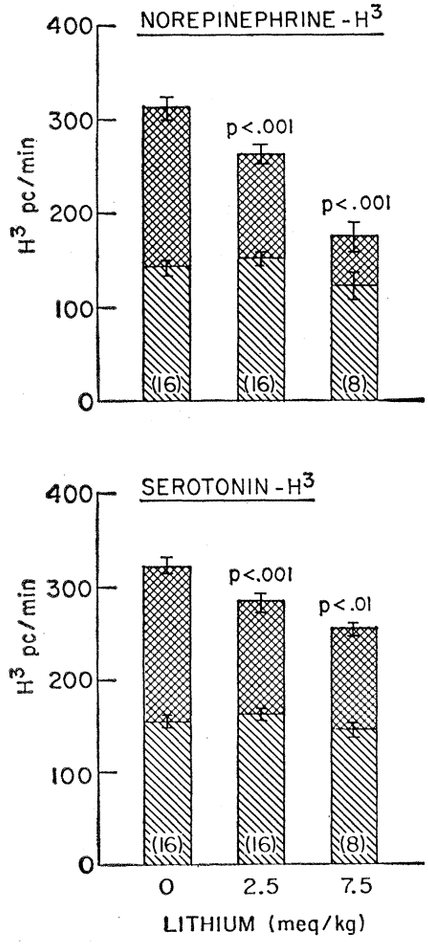

Fig. 2. Effect of lithium treatment in vivo on monoamine release. Striatal slices of rats that received prior treatment with lithium were incubated with tritiated amines, superfused, and stimulated. Results are presented as in Fig. 1. Doses of lithium given to the rats are shown on the abscissa; "0" refers to saline control.

compounds. Stimulation-induced release, however, was significantly reduced (Fig. 1). When other monovalent cations (cesium, rubidium, choline, or tetramethylammonium, all 2.4 meq/liter) were substituted for lithium in the perfusion medium, no differences in spontaneous or evoked release could be demonstrated. Stimulation-induced amine release from brains of rats treated with lithium in vivo intraperitoneally for 3 days (2.5 or 7.5 meq/kg) was also significantly diminished (Fig. 2). This diminution was more marked in rats that had received the higher dose.

Schildkraut *et al.* (*4*) found that lithium (1.2 meq/kg) alters the metabolism of intracisternally injected H^3-norepinephrine. There was a decrease in *O*-methylated metabolites and a concomitant increase in deaminated metabolites, suggesting that less norepinephrine was released in active form. Corrodi *et al.* (*5*), using higher doses of lithium (2.5 to 15 meq/kg), reported that prior treatment with this ion enhanced the decrease in brain norepinephrine induced by an inhibition of tyrosine hydroxylase. We have found that lithium treatment, both in vivo and in vitro at concentration of 2.4 meq/liter (a concentration comparable to that found in the brain 8 hours after intraperitoneal injection of 7.5 meq/kg), diminishes electrically induced release of both H^3-norepinephrine and H^3-serotonin from brain slices previously incubated with these amines.

These results are not in conflict. Acceleration of norepinephrine turnover, suggested by the results reported by Corrodi *et al.* (*5*), may be a consequence of increased intraneuronal destruction by monoamine oxidase. The findings of Schildkraut *et al.* (*4*) support the view that destruction by monoamine oxidase is enhanced, and our results indicate that amine release is diminished. Since newly synthesized norepinephrine is preferentially released (*12*), turnover rates may reflect primarily intraneuronal metabolism. Turnover is therefore not equivalent to synthesis (*13*), and a decrease in release could be accompanied by an increase in intraneuronal metabolism and turnover.

The ability of lithium to inhibit monoamine release from brain slices is consistent with the changes in amine metabolism previously reported and provides further support for the hypothesis that abnormal monoamine metabolism attends certain affective disorders. The effect of lithium on more than one potential neurotransmitter may account in part for the multiple psychopharmacologic actions of this agent.

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References

1. J. J. Schildkraut, S. M. Schanberg, G. R. Breese, I. J. Kopin, *Amer. J. Psychiat.* **124**, 600 (1967); W. E. Bunney, Jr., and J. Davis, *Arch. Gen. Psychiat.* **13**, 483 (1965).
2. J. Glowinski and J. Axelrod, *J. Pharmacol. Exp. Therap.* **149**, 43 (1965); A. Coppen, *Brit. J. Psychiat.* **113**, 1237 (1967).
3. S. Gershon and A. Yuwiler, *J. Neuropsychiat.* **1**, 229 (1960); M. Schou, in *Antidepressant Drugs*, S. Garattini and M. N. G. Dukes, Eds. (Excerpta Medica Foundation, Amsterdam, 1967), p. 80; P. Baastrup and M. Schou, *Arch. Gen. Psychiat.* **16**, 162 (1967).
4. J. J. Schildkraut, S. M. Schanberg, I. J. Kopin, *Life Sci.* **5**, 1479 (1966).
5. H. Corrodi, K. Fuxe, T. Hokfelt, M. Schou, *Psychopharmacologia* **11**, 345 (1967).
6. R. J. Baldessarini and I. J. Kopin, *J. Pharmacol. Exp. Ther.* **156**, 31 (1966); R. J. Baldessarini and I. J. Kopin, *Science* **152**, 1630 (1966).
7. T. N. Chase, G. R. Breese, I. J. Kopin, *Science* **157**, 1461 (1967).
8. H. Dengler, I. Michaelson, H. Spiegel, E. Titus, *Int. J. Neuropharmacol.* **1**, 23 (1962).
9. S. Ross and A. Renyi, *Life Sci.* **6**, 1407 (1967); K. Blackburn, P. French, R. Merrills, *ibid.*, p. 1653.
10. L. Stjarne, *Pharmacol. Rev.* **18**, 425 (1966).
11. I. M. Gibson and H. McIlwain, *J. Physiol. (London)* **176**, 261 (1965).
12. I. J. Kopin, G. R. Breese, K. R. Krauss, V. K. Weise, *J. Pharmacol. Exp. Ther.*, in press.
13. G. C. Sedvall, V. K. Weise, I. J. Kopin, *ibid.* **159**, 274 (1968).

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Palladium Dichloride Whiskers: Preparation and Properties

Abstract. *Palladium dichloride whiskers can be prepared by reacting palladium with chlorine at temperatures above 500°C. The crystals measure 1 × 100 micrometers and are strong (5 percent elastic deformation). They may be a morphological form of one of the high-temperature polymorphs of $PdCl_2$.*

During the course of early attempts to grow palladium whiskers (*1*) by the thermal decomposition of $PdCl_2$, the growth of $PdCl_2$ whiskers was also observed. These $PdCl_2$ whiskers were insoluble in water and could be exposed to the atmosphere for days without hygroscopic attack, in contrast to Sidgwick's observation that $PdCl_2$ is quite soluble and hygroscopic (*2*).

Soulen and Chappell (*3*), using differential thermal analysis, showed that (i) crystalline transitions of $PdCl_2$ occur at 400° and 500°C and (ii) the intermediate form slowly reverts (in the order of months) to the more familiar form which consists of a very long chain at room temperature (*4*). Reconsideration of the conditions under which $PdCl_2$ whiskers can be grown strongly suggests that they may consist of one of these high-temperature polymorphs.

The apparatus used to study the growth of whiskers at high temperature is described elsewhere (*5*). Decompositions of samples (0.5 g) of $PdCl_2$ in Vycor boats with a stream of flowing argon (about 0.4 cm/sec) at 960°C usually produce a massive substrate of palladium crystals from which grow numerous clusters of palladium whiskers 1 to 10 mm long (*1*). Webb has described their exact growth conditions in greater detail (*6*).

Palladium dichloride crystals grow if the reaction boat is removed from the hot zone immediately after the thermal decomposition of liquid $PdCl_2$. Apparently, residual chlorine in the cooler, downstream end of the reaction tube reacts with the hot palladium in the reaction boat to yield masses of well-formed $PdCl_2$ whiskers and filaments. Several types of $PdCl_2$ whiskers include (i) closely connected masses of thousands of filaments and whiskers (up to 1 mm long) that cover the general area where palladium whiskers grow; (ii) large numbers of individual $PdCl_2$ whiskers (about 0.1 mm long) that grow on the sides of the palladium whiskers (Fig. 1); and (iii) numerous loose clusters of $PdCl_2$ whiskers (0.1 mm long) that are far removed from the original palladium metal.

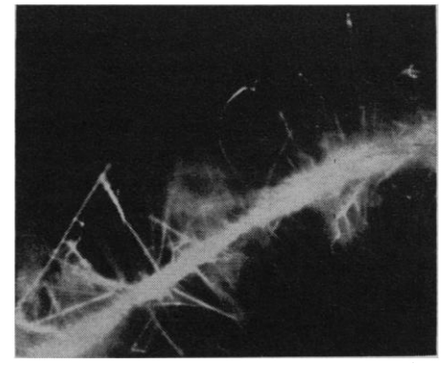

Fig. 1. Palladium dichloride whiskers (0.1 mm long) on a palladium whisker. The salt whisker under strain is withstanding an elastic deformation of about 2.5 percent.