manner preferentially blocks activity in A fibers over that in C fibers (*12*), these data further support the view that hyperalgesia is signaled by activity in A fibers. Since pain sensation in uninjured skin was not attenuated by the block, C-fiber conduction evidently is not interrupted by this block and the pain evoked is probably signaled by activity in the C fibers.

These results indicate that C-fiber nociceptive afferents code for the intensity of thermal pain near pain threshold (43° to 48°C) on the glabrous skin of the uninjured hand. Above 48°C, myelinated nociceptive afferents also contribute to pain sensation. Their activity accounts for the pain during a prolonged, intense heat stimulus and for the hyperalgesia that results minutes after a burn (*13*).

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References and Notes

1. P. Bessou and E. R. Perl, *J. Neurophysiol.* **32**, 1025 (1969); E. R. Perl, T. Kumazawa, B. Lynn, P. Kenins, *Prog. Brain Res.* **43**, 263 (1976); R. E. Beitel and R. Dubner, *J. Neurophysiol.* **39**, 1160 (1976); T. Kumazawa and E. R. Perl, *ibid.* **40**, 1325 (1977); B. Lynn, *J. Physiol. (London)* **287**, 493 (1979); M. Fitzgerald, *ibid.* **297**, 207 (1979); J. G. Thalhammer and R. H. LaMotte, paper presented at the Ninth Annual Meeting of the Society for Neuroscience, Atlanta, 1979.
2. J. N. Campbell, R. A. Meyer, R. H. LaMotte, *J. Neurophysiol.* **42**, 1669 (1979).
3. M. Fitzgerald and B. Lynn, *J. Physiol. (London)* **365**, 549 (1977).
4. In the neurophysiological experiments stimuli were applied to a spot in the center of the fiber's receptive field. The potential contribution of afferents having receptive fields adjacent to or partially overlapping the stimulated spot was not studied.
5. R. A. Meyer, R. E. Walker, V. B. Mountcastle, *IEEE Trans. Biomed. Eng.* **23**, 54 (1976).
6. S. S. Stevens and E. H. Gallanter, *J. Exp. Psychol.* **54**, 377 (1957).
7. R. H. LaMotte and J. N. Campbell, *J. Neurophysiol.* **41**, 509 (1978).
8. Other burn protocols that have been used include application of 53°C heat for 3 or 20 seconds, 49°C for 20 seconds, and 49°C for 1 minute. Following these stimuli the AMH's consistently showed an enhanced response whereas the CMH's consistently showed a suppressed response. Some of these results were reported previously (*2, 7*).
9. The AMH's had a conduction velocity of 34.7 ± 1.8 m/sec (mean ± S.E.; $N = 58$), a receptive field area of 37.3 ± 2.8 mm^2 ($N = 59$), and a mechanical threshold of 3.60 ± 0.26 bars ($N = 62$). The CMH's had a conduction velocity of 0.81 ± 0.05 m/sec ($N = 45$), a receptive field area of 20.8 ± 1.8 mm^2 ($N = 67$), and a mechanical threshold of 5.35 ± 0.44 bars ($N = 67$). These data are consistent with data we reported previously (*2, 7*).
10. The psychophysical data were normalized by dividing each subject's rating for a given stimulus by his rating for the initial 45°C stimulus and then averaged across subjects. The total impulse count during the stimulus interval was used as a measure of the response for the nerve fibers. The CMH data were normalized by dividing each fiber's response by the average response of the CMH's to the initial 45°C stimulus and then averaged across fibers. Since most AMH's did not respond to the initial 45°C stimulus, the AMH data were normalized by dividing each fiber's response by the average response of the AMH's to the first 45°C stimulus after the burn and then averaged across fibers.
11. Two 45°C stimuli were presented during the test sequence. Since the first 45°C stimulus was used to normalize the data, only the response to the second presentation of the 45°C stimulus is shown in Fig. 1. The decreased response to the second 45°C stimulus relative to the response to the first 45°C stimulus (as indicated by normalized values less than 1.0 for the response to 45°C shown in Fig. 1) was reported previously (*7*).
12. S. Sunderland, *Nerves and Nerve Injuries* (Churchill Livingstone, New York, 1978), p. 72.
13. Our preliminary experiments in other areas of the body indicate that these conclusions for glabrous skin may not have general applicability. Following a 53°C, 30-second burn to the hairy skin of seven human subjects, the threshold for pain decreased but the responses to stimuli above 46°C were not significantly changed. Although AMH's innervating the hairy skin became sensitized, the relative density of AMH's in hairy skin appears to be substantially lower than in glabrous skin. In addition, we found that CMH's innervating hairy skin show signs of sensitization not only after the burn stimulus but also after the test stimuli. It is not known whether the mechanism for hyperalgesia proposed here holds for other types of injury, such as mechanical insults, ultraviolet irradiation, and burns caused by less intense heat.
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Lithium Increases Serotonin Release and Decreases Serotonin Receptors in the Hippocampus

Abstract. *The effects of long-term lithium administration on pre- and postsynaptic processes involved in serotonergic neurotransmission were measured in rat hippocampus and cerebral cortex. Long-term lithium administration increased both basal and potassium chloride–stimulated release of endogenous serotonin from the hippocampus but not from the cortex. Serotonergic receptor binding was reduced in the hippocampus but not in the cortex. These results suggest a mechanism by which lithium may stabilize serotonin neurotransmission.*

Lithium is the most specific drug used for the treatment and prevention of recurrent manic-depressive disorders (*1*). The molecular mechanisms related to the therapeutic actions of lithium are not known, but determination of the neuronal effects of this ion may help to elucidate the regulation of neurotransmission and improve our understanding of the pathophysiological processes underlying affective disorders.

Alterations in neurotransmission at serotonin (5HT) synapses have been implicated in affective disorders, and lithium has been reported to affect several serotonergic processes, including synthesis (*2, 3*), release (*4*), and uptake of 5HT (*5*). Long-term administration of lithium exerts specific effects on the binding of [^{3}H]5HT to receptors in the rat brain (*6, 7*), resulting in a reduction of the density of [^{3}H]5HT binding sites in the hippocampus, but not in the cerebral cortex (*6*).

Two types of 5HT receptors can be distinguished in the rat brain: $5HT_1$ receptors to which [^{3}H]5HT binds and $5HT_2$ receptors in the cortex and possibly in the hippocampus to which [^{3}H]-spiperone binds (*8, 9*). We studied the effects of long-term administration of lithium on both [^{3}H]5HT and [^{3}H]spiperone receptor binding sites and on release and uptake of 5HT in rat cortex and hippocampus. We report that long-term administration of lithium exerts brain region specific effects on both types of 5HT receptor binding sites and on the release of endogenous 5HT.

Male Sprague-Dawley rats (220 to 240 g) were housed five to a cage in a light-controlled room (12 hours light, 12 hours dark; lights on at 7 a.m.) with temperature maintained at 22°C. The experimental groups were fed a diet containing 0.24 percent lithium carbonate mixed in powdered rat food. Control groups were fed the powdered food without added lithium. Both groups had water and hypertonic saline (0.46*M*) available to drink at all times. After being on the diet for 4 to 6 weeks, the rats were decapitated, and serum lithium and sodium concentrations were measured by flame photometry. The brain was dissected over ice and was either used immediately for studies of the uptake of [^{3}H]5HT and the release of endogenous 5HT or was frozen at −80°C for later study of the binding of [^{3}H]5HT and [^{3}H]spiperone to receptors.

Serum lithium concentrations in the experimental groups ranged from 0.9 to 1.1 meq/liter. There was no significant difference in serum sodium between the groups receiving lithium and the control groups. The lithium-treated rats gained weight more slowly than the controls, but appeared healthy; however, the lithium-treated rats showed the polyuria and polydipsia that are associated with therapeutic concentrations of lithium.

Serotonin receptor binding was measured in homogenate fractions after two centrifugations at 49,000*g* for 10 min-

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Table 1. Effects of long-term lithium administration on pre- and postsynaptic serotonergic processes. Values are means ± standard error for the number of animals shown in parentheses. Basal release values were calculated from the release period immediately preceding stimulation. Stimulated release values were calculated from the first stimulation period.

Item	Hippocampus		Cortex	
	Control	Lithium	Control	Lithium
[^{3}H]5HT bound* (pmole per gram of tissue)	8.0 ± 0.5 (10)	5.8 ± 0.4† (10)	6.8 ± 0.3 (10)	6.7 ± 0.4 (10)
[^{3}H]Spiperone bound (pmole per gram of tissue)	4.7 ± 0.4 (5)	3.7 ± 0.2† (5)	10.7 ± 0.4 (5)	10.0 ± 0.6 (5)
[^{3}H]5HT uptake (pmole per 4 mg of tissue in 5 minutes)	1.32 ± 0.4 (5)	1.29 ± 0.04 (5)	1.31 ± 0.08 (5)	1.23 ± 0.09 (5)
Basal 5HT release (fmole per milligram of protein in 2 minutes)	88.6 ± 2.6 (6)	120.2 ± 3.8† (6)	35.2 ± 4.4 (6)	43.4 ± 5.6 (6)
Stimulated 5HT release (fmole per milligram of protein in 2 minutes)	157.8 ± 19.5 (6)	216.2 ± 16.4† (6)	78.5 ± 3.9 (6)	68.2 ± 6.3 (6)
5HT content (pmole per milligram of protein)	25.9 ± 2.6 (5)	16.8 ± 1.3† (5)	26.8 ± 2.5 (5)	23.4 ± 1.2 (5)

*Data from (*6*). †$P < .05$, in comparison with control values.

utes, followed by washing. Portions of tissue equivalent to 8 mg of cortex or hippocampus were assayed. For measurement of binding of [^{3}H]5HT to $5HT_1$ receptors, tissues were incubated for 5 minutes at 37°C in 50 m*M* tris-HCl buffer (*p*H 7.4, 25°C) containing 4 m*M* $CaCl_2$, 0.1 percent ascorbic acid, and 10 μ*M* pargyline; [^{3}H]5HT (5.0 n*M*) was then added, and the tissues were incubated for 10 minutes at 37°C. Assays were carried out in sextuplicate; half of the tubes contained 10 μ*M* unlabeled 5HT for measurement of nonspecific binding. The binding of [^{3}H]spiperone (1.0 n*M*) to $5HT_2$ sites was carried out at 37°C for 10 minutes in 50 m*M* tris-HCl buffer (*p*H 7.4, 25°C) containing 0.1 percent ascorbic acid. Assays were carried out in sextuplicate; half of the tubes contained 10 μ*M* (+)-butaclamol for measurement of nonspecific binding. All reactions were terminated by the addition of 4 ml of ice-cold buffer to each tube and rapid filtration of the samples under reduced pressure (Whatman GF/B glass fiber filters). The tubes and filters were then rapidly washed three times with cold buffer. The radioactivity retained in the filters was counted by liquid scintillation spectrometry. Specific binding, defined as the difference between values for binding in the absence and in the presence of saturating concentrations of nonradioactive ligand, was 65 to 75 percent for [^{3}H]5HT binding in the cortex and hippocampus, 55 to 60 percent for [^{3}H]spiperone binding in the cortex, and 35 to 50 percent for [^{3}H]spiperone binding in the hippocampus, where the density of these sites is much less than that in the cortex.

The active uptake of [^{3}H]5HT into brain homogenates was studied in fresh cortex and hippocampus. Tissues were homogenized in ice-cold 0.32*M* sucrose and centrifuged at 900*g* for 10 minutes. Portions of supernatant equivalent to 4 mg of tissue were added to oxygenated Krebs phosphate buffer (*p*H 7.35), which also contained 10 m*M* glucose, 1.1 m*M* ascorbic acid, and 75 μ*M* nialamide. The tissues were incubated for 4 minutes at 37°C, and then [^{3}H]5HT (0.05 μ*M*) was added, and the tubes were incubated for 5 minutes at 37°C. Identical tubes kept in an ice-water bath at 0°C throughout the procedure served as blanks. The assays were terminated by adding 2 ml of ice-cold buffer to each tube and filtering the samples under reduced pressure through Millipore filters (pore size, 0.3 μ*M*). The filters were washed three times with ice-cold buffer, transferred to counting vials, dissolved in liquid scintillation solution, and counted in a liquid scintillation counter. Active uptake, defined as the difference in [^{3}H]5HT accumulated in the tissue at 37° and 0°C, was more than 90 percent of the total uptake.

Release of endogenous 5HT was measured in slices of fresh hippocampus and frontal cortex (frontal third) by the method of Wenthold (*10*). Tissue slices were suspended in a bicarbonate buffer containing 20 m*M* $NaHCO_3$, 140 m*M* NaCl, 2.5 m*M* $CaCl_2$, 3.5 m*M* KCl, 1 m*M* $MgSO_4$, and 3.3 m*M* glucose. The *p*H was maintained at 7.4 by bubbling with 95 percent O_2 and 5 percent CO_2. The procedure for measuring release included incubation of slices at 37°C for 2 minutes, centrifugation for 30 seconds, removal of the supernatant for analysis, and resuspension of the tissue slices in fresh buffer. Tissues were incubated for 8 to 12 minutes with four to six changes of buffer to allow the basal release to stabilize. Release was then measured at 2-minute intervals, either in normal bicarbonate buffer (basal release) or in bicarbonate buffer containing 50 m*M* KCl with equimolar reduction of NaCl (stimulated release). Basal release was measured before and after KCl-stimulat-

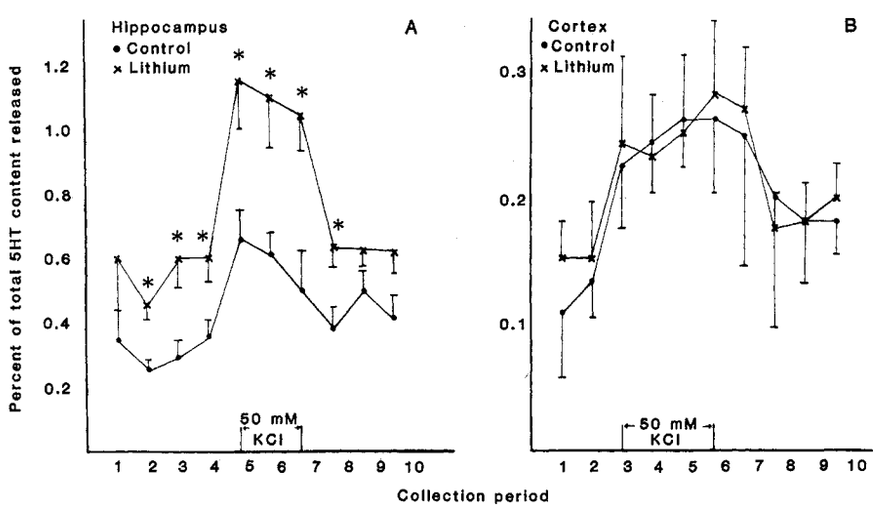

Fig. 1. Release of endogenous serotonin from slices of (A) the hippocampus and (B) the cortex from control rats and rats treated with lithium for 5 weeks. Collections were made at 2-minute intervals after basal release stabilized. Endogenous serotonin released into the medium was assayed by the method of Saavedra *et al.* (*11*). The amount released during each 2-minute period was expressed as a percent of the total serotonin content of the tissue. Stimulated release in the presence of 50 m*M* KCl was measured during the periods indicated on the abscissa. Each point is the mean ± standard error for five animals. The asterisk represents $P < .05$ compared with control values.

ed release. The supernatant from each collection was immediately acidified in 0.1*N* HCl and frozen until assayed. At the end of the assay, tissue slices were homogenized in 0.1*N* HCl and frozen. The 5HT content of each sample was assayed by radioenzymatic methods (*11*). The data were analyzed by expressing the 5HT released in each sample both as a fraction of total 5HT content and as the amount of transmitter released per milligram of protein. In all experiments, control and lithium-treated groups were assayed in parallel, and data were analyzed for statistical significance by Student's *t*-test.

Long-term administration of lithium reduced the binding of both [^{3}H]5HT and [^{3}H]spiperone in the hippocampus (Table 1). In the cortex neither of these binding sites appeared to be affected (Table 1). The addition of lithium in vitro in a concentration range of 0.5 to 10.0 meq/liter had no effect on [^{3}H]5HT or [^{3}H]spiperone binding sites. Therefore, the lithium-induced decreases in these binding sites in the hippocampus are brain region specific and due to pharmacological effects in vivo.

Identification of [^{3}H]spiperone binding sites in the hippocampus as $5HT_2$ receptors is still tentative. Recent reports indicate that only a small percentage of these binding sites are serotonergic, and the remainder may be binding sites for spirodecanone structures (*12*).

To determine whether these effects on receptors were related to presynaptic serotonergic processes, we examined the effects of long-term lithium administration on the uptake and release of 5HT. Long-term lithium administration had no effect on [^{3}H]5HT uptake in either cortex or hippocampus (Table 1). However, lithium treatment produced marked brain region specific effects on the release of endogenous 5HT. In the hippocampus, lithium administration increased both basal and K^+-stimulated release (Fig. 1A). The net fractional stimulated release (stimulated release minus basal release) from the hippocampus of lithium-treated rats was twice that from the hippocampus of control rats. Expressed on a molar basis, lithium administration induced a 36 percent increase in both basal and K^+-stimulated release and a 39 percent increase in net stimulated release from the hippocampus (Table 1). Thus, long-term administration of lithium resulted in the release of a greater fraction of total 5HT stores and a greater amount of 5HT that could act on receptors in the hippocampus. In contrast, neither fractional release of 5HT nor the amount of 5HT released from cortical slices appeared to be affected by prior administration of lithium (Fig. 1B and Table 1). In the hippocampus, the 5HT content was significantly decreased after long-term lithium administration (Table 1). This may be a reflection of increased 5HT release in vivo, since no effect on 5HT content in the cortex was seen (Table 1).

These findings indicate that long-term lithium administration produces brain region specific effects on 5HT neurotransmission processes and that the effects of lithium on 5HT receptors can be correlated to actions on 5HT release. It is not yet known whether the decrease in 5HT receptors is a result of an increase in 5HT release or whether the primary effect of lithium is postsynaptic, the increase in 5HT release being an adaptive response to decreased receptor binding sites.

Our data on lithium release appear to contrast with earlier reports that lithium decreased stimulated release of previously loaded [^{3}H]5HT (*4*). However, those studies were carried out on slices of corpus striatum from animals that had been treated with lithium for only 3 days or on slices incubated with lithium in vitro. In studies measuring whole brain content, lithium was reported to have no significant effect on 5HT levels (*13*). Other investigators found that long-term administration of lithium decreases 5HT content in specific brain areas (*2*). We found a significant decrease in 5HT content in the hippocampus, but not in the frontal cortex.

These results indicate that lithium has pronounced effects on the release and receptor components of serotonergic neurotransmission processes in the hippocampus. Lithium also increases the transport of the 5HT precursor tryptophan into the hippocampus (*14*) and striatum (*3, 14, 15*), but not into the cortex (*14*). Lithium administration increases tryptophan uptake into slices of rat striatum, and short-term administration of lithium increases the synthesis of 5HT from tryptophan by the enzyme tryptophan hydroxylase (*16*). With long-term administration, the synthesis rate of 5HT returns to control values because of a decrease in tryptophan hydroxylase (*16*). The decrease in tryptophan hydroxylase is hypothesized to be a compensatory response to an initial and transitory increase in 5HT release (*17*). According to this model (*17*), after long-term administration of lithium, synthesis and release return to control values, but 5HT synthesis is protected against further changes and serotonergic neurotransmission is stabilized.

Our data are consistent with the concept that lithium stabilizes serotonergic neurotransmission, but suggest that other mechanisms are responsible. Release, rather than returning to control levels after chronic administration of lithium, is increased in the hippocampus while serotonergic receptors are decreased. Thus there may be no net change in 5HT neurotransmission, but the increased activity of the release process coupled with the decrease in receptors may render 5HT neurotransmission resistant to further changes. These effects on 5HT neurotransmission might then account for part of the effectiveness of lithium in manic-depressive disorders.

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References and Notes

1. F. K. Goodwin and M. H. Ebert, in *Lithium: Its Role in Psychiatric Research and Treatment*, S. Gershon and B. Shopsin, Eds. (Plenum, New York, 1973), p. 237.
2. A. K. S. Ho, H. H. Loh, F. Craves, R. J. Hitzemann, S. Gershon, *Eur. J. Pharmacol.* **10**, 72 (1970).
3. S. Knapp and A. J. Mandell, *Science* **180**, 645 (1973).
4. R. I. Katz, T. N. Chase, I. J. Kopin, *ibid.* **162**, 466 (1968); R. I. Katz and I. J. Kopin, *Biochem. Pharmacol.* **18**, 1935 (1968).
5. E. F. Friedman and S. Gershon, *Int. Congr. Pharmacol.* **1978**, 855 (Abstr.) (1978).
6. S. Treiser and K. J. Kellar, *Eur. J. Pharmacol.* **64**, 183 (1980).
7. A. Maggi and S. J. Enna, *J. Neurochem.* **34**, 888 (1980).
8. S. J. Peroutka and S. H. Snyder, *Mol. Pharmacol.* **16**, 687 (1979).
9. I. Creese and S. H. Snyder, *Eur. J. Pharmacol.* **49**, 201 (1978).
10. R. J. Wenthold, *Brain Res.* **162**, 338 (1979).
11. J. M. Saavedra, M. Brownstein, J. Axelrod, *J. Pharmacol. Exp. Ther.* **186**, 508 (1973).
12. D. R. Howlett, H. Morris, S. R. Nahorski, *Mol. Pharmacol.* **15**, 506 (1979); J. M. Palacios, D. L. Niehoff, M. J. Kuhar, *Brain Res.* **213**, 277 (1981).
13. E. L. Bliss and J. Ailion, *Brain Res.* **24**, 305 (1970).
14. A. C. Swann *et al.*, *ibid.* **194**, 287 (1980).
15. S. Knapp and A. J. Mandell, *Life Sci.* **18**, 679 (1976).
16. ———, *J. Pharmacol. Exp. Ther.* **193**, 812 (1975).
17. A. J. Mandell, in *Impact of Biology on Modern Psychiatry*, E. S. Gershon, R. H. Belmaker, S. S. Kety, M. Rosenbaum, Eds. (Plenum, New York, 1977), p. 165.
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