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BRIEF COMMUNICATION

Unchanged Sensitivity to Electric Shock in L-Tryptophan Treated Rats

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HOLE, K. AND C. A. MARSDEN. *Unchanged sensitivity to electric shock in L-tryoptophan treated rats.* PHARMAC. BIOCHEM. BEHAV. 3(2) 307–309, 1975. – Injections of L-tryptophan (50, 100 and 200 mg/kg) i.p. in rats resulted in dose dependent increases in brain concentrations of tryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. Sensitivity to electric shock as measured by flinch and jump thresholds was not changed by L-tryptophan 100 and 200 mg/kg. The results indicate that increased 5-HT turnover does not influence sensitivity to electric shock.

Shock sensitivity Tryptophan 5-Hydroxytryptamine Morphine

SEVERAL previous experiments have suggested that serotonergic neurons play an important role in central control of pain sensitivity. Jump threshold is reduced both by lesions of the medial forebrain bundle [7] and by parachlorophenylalanine treatment [9,16]. Since injections of 5-hydroxytryptophan (5-HTP) normalize jump threshold in both these preparations, it has been proposed that a defective function of serotonergic neurons increases pain sensitivity [7,16].

It also has been proposed that electrical stimulation of serotonergic neurons [1] and increase in brain 5-hydroxytryptamine (5-HT) concentration [16] decrease pain sensitivity, and that the analgesic effect of morphine is due to its ability to increase brain 5-HT turnover [15, 19, 21]. A diurnal variation in jump threshold in parallel to the diurnal variation in brain 5-HT concentration also has been observed (J. A. Harvey, personal communication to R. J. Wurtman [20]). 5-HTP administration to normal animals, however, does not decrease sensitivity to electric shock [7,16]. Since 5-HTP (unlike tryptophan) administration results in an abnormal distribution of the 5-HT formed [4,14], the interpretation of these results is unclear. The aim of the present experiment was to investigate whether an increase in brain 5-HT concentration and turnover produced by administration of L-tryptophan affects sensitivity to electric shock as measured by flinch and jump thresholds.

METHOD

Animals

Male wistar rats (n = 46) weighing 240–360 g were used. They were injected with L-tryptophan (50, 100 and 200 mg/kg body weight i.p.) given as a suspension in 1% carboxymethylcellulose dissolved in 0.9% NaCl. Controls were injected with the vehicle. Separate groups of rats were used for behavioural testing and for biochemical estimations.

Procedure

Flinch-jump test. Testing was started 1 hr after tryptophan injection. The test has been described in detail elsewhere [9]. Briefly, electric shocks of 0.2 sec duration were delivered to a grid floor from a Grason-Stadler shock generator at intervals of 15–45 sec. The rat's response to each shock was scored. Intensity of the first shock was 0.05 mA, increasing for each shock until a flinch (crouch or startle, lifting one front paw) was elicited. Intensity of the shock was decreased by one step whenever a flinch was scored, and increased by one step when a flinch was not observed. When 8 flinches had been scored, shock intensity was increased until a jump (both rear paws leaving the grid floor simultaneously, or stepping vigorously) was elicited. Testing was ended when a jump had been scored 8 times, shock

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intensity being increased or decreased in a similar way as during flinch testing. Flinch and jump thresholds were the medians of the 8 values.

Biochemical assays. Rats were killed by guillotine 1 hr after administration of tryptophan. The brain was rapidly removed, the cerebellum discarded and the rest of the brain frozen on dry ice and stored at −20°C. Brain tryptophan, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were determined as described by Curzon, Joseph and Knott [3].

RESULTS

L-Tryptophan (50, 100 and 200 mg/kg) administration produced a dose dependent, linear, increase in brain tryptophan concentrations ($r = 0.82$, $p<0.001$; Fig. 1). There also was a linear increase in 5-HT turnover ($r = 0.89$), $p<0.001$; Fig. 1) when the concentration of total 5-hydroxyindoles (5-HT + 5-HIAA) is used as an index of turnover [14].

There was no significant change, however, in either the flinch or jump threshold after either 100 or 200 mg/kg L-tryptophan (Mann-Whitney U-test) (Table 1).

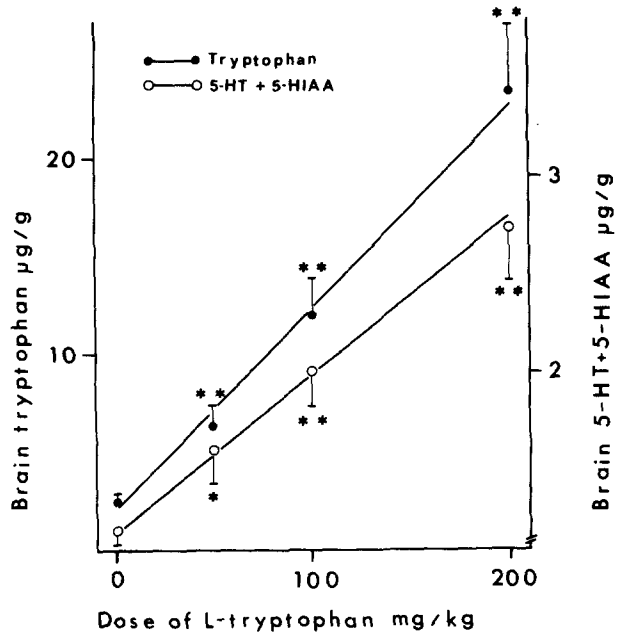

FIG. 1. Brain concentrations of tryptophan and total 5-hydroxyindoles (5-HT + 5-HIAA), mean ± SD. L-Tryptophan (0, 50, 100 or 200 mg/kg) injected i.p. 1 hr before killing, n = 6 for each dose level. *$p<0.05$, **$p<0.001$ compared with controls (t test).

DISCUSSION

L-tryptophan administration clearly increased brain 5-HT turnover. Since 5-HT formation from tryptophan takes place within serotonergic neurons [14], release of transmitter and stimulation of serotonergic receptors probably were increased, although at least part of the 5-HT formed may not reach the functional pool [5].

The increase in 5-HT formation above normal levels did not change the sensitivity to electric shock as the flinch and jump thresholds were unchanged. This indicates that increases in turnover only are not sufficient to produce analgesia. The results therefore do not support the hypothesis that the analgesic effect of morphine may be due to an increase in brain 5-HT turnover (12, 15, 21], but agree with studies showing no relationship between morphine's analgesic effect and serotonergic function [2,11].

TABLE 1

MEAN FLINCH AND JUMP THRESHOLDS IN l-TRYPTOPHAN TREATED RATS

Tryptophan dose, mg/kg*	Flinch mA ± SD	Jump mA ± SD
0 (8)	0.11 ± 0.04	0.39 ± 0.10
100 (6)	0.12 ± 0.03	0.43 ± 0.10
200 (8)	0.11 ± 0.02	0.41 ± 0.09

*Injected i.p. 1 hr before testing started. No. of rats in parentheses.

Possibly an increase in 5-HT formation can reduce pain sensitivity only when this sensitivity is abnormally high, as in the medial forebrain bundle lesioned or parachlorophenylalanine treated animals [8]. Strain differences may account for some of the discrepancies in the literature [17]. However, it also seems possible that serotonergic neurons have no particular function in the control of pain sensitivity. When serotonergic neurons are destroyed by selective electrolytic lesions in the dorsal and median raphe nuclei, or chemically by injection of 5,7-dihydroxytryptamine in the raphe nuclei [9] or 5,6-dihydroxytryptamine intraventricularly [2,18], no increase in pain sensitivity is observed. The suggestion that serotonergic neurons are important for control of pain sensitivity is based on experiments where the function of serotonergic neurons has been manipulated by methods that are not specific for these neurons. Thus, electrolytic lesions in the medial forebrain bundle [7] and in the midbrain raphe region [2] also destroy non-serotonergic neurons. Parachlorophenylalanine treatment [9,16] reduces brain 5-HT concentrations but also brain catecholamines to some extent [10], and inhibits transport into the brain of amino acids [6]. Electrical stimulation in the midbrain raphe region [1,13] stimulates not only 5-HT neurons, but also non-serotonergic neurons. Similarly 5-HTP treatment increases 5-HT formation within 5-HT neurons, but also leads to the formation of 5-HT within catecholamine neurons [4]. Harvey and Yunger [8] recently suggested that the reversal of hyperalgesia in lesion animals produced by 5-HTP may be due to accumulation of 5-HT in catecholaminergic terminals.

Thus at present there seems to be no clear evidence for a relationship between decreased activity in serotonergic neurons and increased pain sensitivity, or from the present experiment, increased brain 5-HT turnover and decreased sensitivity to electric shock. It is important, however, to determine to what extent the L-tryptophan induced increase in 5-HT influences the function of 5-HT neurons and receptors.

REFERENCES

1. Akil, H. and D. J. Mayer. Antagonism of stimulation-produced analgesia by p-CPA, a serotonin synthesis inhibitor. *Brain Res.* **44:** 692–697, 1972.
2. Bläsig, J., K. Reinhold and A. Herz. Effect of 6-hydroxydopamine, 5,6-dihydroxytryptamine and raphe lesions on the antinociceptive actions of morphine in rats. *Psychopharmacologia* **31:** 111–119, 1973.
3. Curzon, G., M. H. Joseph and P. J. Knott. Effects of immobilization and food deprivation on rat brain tryptophan metabolism. *J. Neurochem.* **19:** 1967–1974, 1972.
4. Fuxe, K., L. L. Butcher and J. Engel. DL-5-Hydroxytryptophan-induced changes in central monoamine neurons after peripheral decarboxylase inhibition. *J. Pharm. Pharmac.* **23:** 420–424, 1971.
5. Grahame-Smith, D. J. Studies in vivo on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. *J. Neurochem.* **19:** 1053–1066, 1971.
6. Grundt, I. K. and K. Hole. p-Chlorophenylalanine treatment in developing rats: Protein and lipids in whole brain and myelin. *Brain Res.* **74:** 269–277, 1974.
7. Harvey, J. A. and C. E. Lints. Lesions in the medial forebrain bundle: Relationship between pain sensitivity and telencephalic content of serotonin. *J. comp. physiol. Psychol.* **74:** 28–36, 1971.
8. Harvey, J. A. and L. M. Yunger. Relationship between telencephalic content of serotonin and pain sensitivity. In: *Serotonin and Behavior*, edited by J. Barchas and E. Usdin. New York: Academic Press, 1973, pp. 179–189.
9. Hole, K. and S. A. Lorens. Response to electric shock in rats: effects of selective midbrain raphe lesions. *Pharmac. Biochem. Behav.* **3:** 1975, in press.
10. Koe, B. K. and A. Weissman. p-Chlorophenylalanine: A specific depletor of brain serotonin. *J. Pharmac. exper. Ther.* **154:** 499–516, 1966.
11. Lorens, S. A. and L. M. Yunger. Morphine analgesia, two-way avoidance, and consummatory behavior following lesions in the midbrain raphe nuclei of the rat. *Pharmac. Biochem. Behav.* **2:** 215–22, 1974.
12. Major, C. T. and B. J. Pleuvry. Effects of α-methyl-p-tyrosine, p-chlorophenylalanine, L-β-(3, 4-dihydroxyphenyl) alanine, 5-hydroxytryptophan and diethyldithiocarbamate on the analgesic activity of morphine and methylamphetamine in the mouse. *Br. J. Pharmac.* **42:** 512–521, 1971.
13. Mayer, D. J. and J. C. Liebeskind. Pain reduction by focal electrical stimulation of the brain: An anatomical and behavioral analysis. *Brain Res.* **68:** 73–93, 1974.
14. Moir, A. T. B. and D. Eccleston. The effects of precursor loading in the cerebral metabolism of 5-hydroxyindoles. *J. Neurochem.* **15:** 1093–1108, 1968.
15. Samanin, R. and L. Valzelli. Increase of morphine-induced analgesia by stimulation of the nucleus raphe dorsalis. *Eur. J. Pharmac.* **16:** 298–302, 1971.
16. Tenen, S. S. The effects of p-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behavior in the rat. *Psychopharmacologia* **10:** 204–219, 1967.
17. Tilson, H. A. and R. H. Rech. The effects of p-chlorophenylalanine on morphine analgesia, tolerance and dependence development in two strains of rats. *Psychopharmacologia* **35:** 45–60, 1974.
18. Vogt, M. The effect of lowering the 5-hydroxytryptamine content of the rat spinal cord on analgesia produced by morphine. *J. Physiol.* **236:** 483–489, 1974.
19. Way, E. L., H. H. Loh and F.–H. Shen. Morphine tolerance, physical dependence, and synthesis of brain 5-hydroxytryptamine. *Science* **162:** 1290–1292, 1968.
20. Wurtman, R. J. In: *Aromatic Amino Acids in the Brain*, Ciba Foundation Symposium 22. Elsevier: Amsterdam, 1974, p. 237.
21. Yarbrough, G. G., D. M. Buxbaum and E. Sanders-Bush. Increased serotonin turnover in the acutely morphine treated rat. *Life Sci.* **10:** 977–983, 1971.