*Pharmacology Biochemistry & Behavior*, Vol. 33, pp. 127–130. © Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

# Chronic Administration of Thyrotropin-Releasing Hormone Enhances the Sensitivity of Lumbar Motoneurons to 5-Hydroxytryptophan in the Rat[1]

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TREMBLAY, L. E. AND P. J. BÉDARD. *Chronic administration of thyrotropin-releasing hormone enhances the sensitivity of lumbar motoneurons to 5-hydroxytryptophan in the rat.* PHARMACOL BIOCHEM BEHAV **33**(1) 127–130, 1989.—The influence of a single intraperitoneal injection or of a three-week intrathecal infusion of thyrotropin-releasing hormone (TRH) was studied on the excitatory effect of DL-5-hydroxytryptophan (5-HTP) (20–100 mg/kg IP) on lumbar motoneurons. This effect was studied by recording the integrated spontaneous electromyographic (EMG) activity of the hindlimb muscles in spinalized rats previously denervated with an intrathecal injection of 5,7-dihydroxytryptamine. A single injection of TRH 10 mg/kg IP caused by itself a stimulation of the EMG activity but failed to modify the subsequent EMG response to 5-HTP one hour or one day later. However, a three-week infusion of TRH in the lumbar subarachnoid space caused a 300% increase in the response to 5-HTP, while denervation alone caused only an increase of 160%. A similar potentiation of the effect of 5-HTP was seen on the Wet Dog Shake phenomenon which we believe is elicited in the forebrain. Therefore, chronic but not acute stimulation of lumbar motoneurons or forebrain structures by TRH appears to facilitate the serotonergic excitating response. This effect of TRH does not appear to necessitate the actual coexistence of 5-HT and TRH in the same nerve fibers.

TRH 5-HT EMG WDS Motoneurone Coexistence

THE spinal cord of the rat is innervated at all levels by 5-hydroxytryptamine-containing fibers which originate in the medullary raphe nuclei (7). Several studies have shown that 5-hydroxytryptamine (5-HT) depolarizes lumbar motoneurons (14,27) and increases spinal reflexes (1) as well as spontaneous electromyographic activity in the hindlimbs (3,4).

It has recently been demonstrated that the 5-HT-containing bulbospinal fibers also contain in addition and in various combinations at least two peptides; the undecapeptide substance P and the tripeptide thyrotropin-releasing hormone (TRH) (6, 9, 12, 13). Many of these descending fibers project to the ventral horn of the spinal cord where nerve endings are seen in close approximation of large motoneurons (8,10). Such a close morphological association naturally raises the possibility of a physiological interaction between 5-HT and the two peptides.

We have recently discussed the modulatory role of substance P on the excitability of lumbar motoneurons in the rat (25). In the present work we have investigated possible interactions between 5-HT and TRH in the lumbar spinal cord of the rat where the two substances coexist in the same fibers.

We have previously established that both 5-hydroxytryptophan (5-HTP) and TRH (2) administered systemically increase in a similar manner the spontaneous EMG activity of the hindlimb muscles in chronically spinalized rats. Such an excitatory effect on the EMG activity of the hindlimbs increases with time after the transection, but is found to be maximal with both substances one day after the transection if the animals have been treated twenty days before with 5,7-dihydroxytryptamine, a neurotoxin to the 5-HT pathways, in the cerebral ventricles. Interestingly, the effect of both 5-HTP and TRH can be abolished by cyproheptadine, a

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5-HT receptor blocker, suggesting that 5-HT and TRH excite motoneurons via the same or closely associated receptors. A TRH recognition site has been demonstrated in the spinal cord by Sharif and Burt (21) and by Prasad and Edwards (19). The number of sites increases after denervation by 5,7-dihydroxytryptamine (22) which could explain the behavioral supersensitivity which we observed (2).

When administered to intact rats, TRH produces in the hour that follows an accumulation of 5-hydroxyindoleacetic acid (5-HIAA) which suggests increased release of 5-HT. On the other hand, when TRH and 5-HTP are administered systemically together only, an additive effect is observed on the EMG activity of the hindlimb muscles (unpublished results).

In the present work we have attempted to explore whether TRH could modulate the effect of 5-HTP. The response to 5-HTP was therefore studied in paraplegic rats after a single dose of TRH known to affect motoneuronal excitability (2) or after a chronic exposure to TRH.

The latter experiment was prompted by our recent observation (24) that denervation supersensitivity to 5-HT or 5-HTP in spinalized rats requires more than the absence of 5-HT. Because of the coexistence of 5-HT and TRH it was thought possible that a chronic continuous exposure of the denervated neurons to TRH would prevent the denervation supersensitivity caused by 5,7-DHT.

## METHOD

A total of 49 female Sprague-Dawley rats (Canadian Breeding Farm, St-Constant, Québec) weighing between 290 and 350 g at the beginning of the experiment were used throughout this study.

A permanent, flexible (PE-10) cannula was introduced under ketamine anesthesia (20 mg/kg IP) in the cisterna magna and lowered in the subarachnoid space to the lumbar area (16). The cannula was fixed to the skull with dental cement. One week later, thirty-nine of the animals received an injection of 200 μg (5 mM) (in 100 μl of Ringer) of 5,7-DHT through the cannula. The drug was administered over five minutes with the help of a Harvard infusion withdrawal pump using a Hamilton syringe. We have previously established that this dose of neurotoxin administered intrathecally under the same experimental conditions results in a 95% depletion of 5-HT in the spinal cord (24). Ten animals received an equal volume of Ringer solution.

Twenty-four hours later, thirteen of the 5,7-DHT-treated rats were selected randomly for the chronic TRH treatment. Under ketamine anesthesia, an Alzet minipump (volume: 2 ml infusion rate 2.5 μl/hour) was placed subcutaneously on the back of the animals, between the scapulae. PE-60 flexible tubing was used to connect the minipump with the intrathecal cannula with the help of epoxy glue. The pump was filled with TRH in 7 rats and with vehicle in six. The concentration of TRH, 8 μg/μl (22 mmol/hour) was chosen in preliminary trials and represents 30% of the intrathecal dose necessary to evoke a maximal EMG response. Nine cannulated, 5,7-DHT-treated rats served as controls for the chronic experiment. The intrathecal infusion was continued for twenty days.

All animals were reanesthetized twenty-one days after 5,7-DHT or vehicle. A complete spinal section was performed at the T-6 level. In the chronic treatment group the pump was taken out and inspected to ensure delivery of the drug. Thereafter, monopolar electrodes with appropriate grounds were installed in the left biceps femoris (flexor muscle) and right quadriceps femoris (extensor muscle) of the thigh and relayed under the skin to the connector on the back of the animal for recording of the spontaneous, raw and integrated electromyographic activity. The EMG signals were amplified (Gould 13-4615-56) integrated (Gould 13-4615-70) and displayed by a pen recorder (Gould 2200). For all experiments the paralysed rats were placed in prone position on a small padded platform with access to food and water and with the hindlimbs hanging through holes.

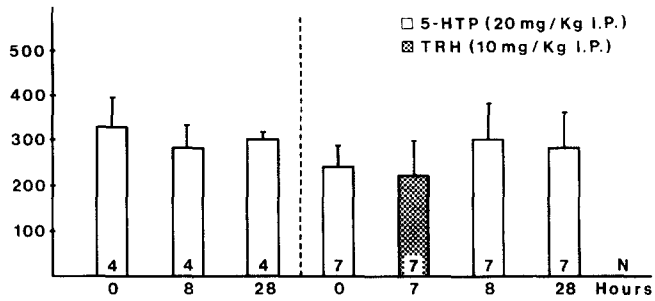


FIG. 1. Graph showing average integrated EMG response ± SEM recorded in the extensor muscles of the thigh of spinalized rats in the thirty minutes following an injection of DL-5-HTP 20 mg/kg IP expressed in percent of the EMG activity recorded in the thirty minutes preceding the injection. The animals had been denervated with 5-7-DHT three weeks before. On the left of the graph is shown the effect of three injections of 5-HTP in the same animals at 0, 8 and 28 hours. A similar experiment is shown on the right, but an injection of TRH 10 mg/kg IP (shaded bar) has been given one hour before the second injection of 5-HTP. The number of animals used is shown in each bar. Statistical comparisons were done with one-way analysis of variance.

The rats in the chronic TRH experiment and their controls (nondenervated or denervated and vehicle-treated) were tested once with DL-5-HTP 100 mg/kg IP twenty-four hours after spinalization.

The animals in the acute experiment were also tested with DL-5-HTP (20 mg/kg IP) twenty-four hours after spinalization but then received a single dose of TRH 10 mg/kg IP seven hours later. They were again tested for their response to 5-HTP one hour and twenty-one hours after TRH. EMG recordings were always done for thirty minutes before and thirty minutes after the test drug. Quantification was achieved by counting the number of peaks on the integrated EMG tracing and comparing the pre and postdrug periods as described previously (4).

Furthermore, in some of the groups, the number of characteristic rhythmic wet dog shakes (WDS) of neck, head and trunk was counted for five-minute periods before and 20 minutes after 5-HTP administration (5).

The following drugs were used: DL-5-hydroxytryptophan (DL-5-HTP) 20 or 100 mg/kg IP, Sigma; thyrotropin-releasing hormone (TRH) 10 mg/kg IP and 22 mmol/hour IT, Peninsula Laboratories; 5,7-dihydroxytryptamine (5,7-DHT) 200 μg/μl, IT, Sigma. TRH and DL-5-HTP were dissolved in saline containing a minimal quantity of HCl (for 5-HTP). 5,7-DHT was dissolved in Ringer solution with ascorbic acid.

## RESULTS

As shown in Fig. 1 the response to the same dose of 5-HTP repeated three times over twenty-four hours is stable in animals spinalized one day previously and denervated with 5,7-DHT three weeks before. As seen on the right of the figure, an injection of TRH 10 mg/kg IP after the first dose of 5-HTP does not modify the response to 5-HTP one hour or one day later.

On the other hand, as seen on Fig. 2, chronic intrathecal treatment with TRH for twenty days increases the motor response to 5-HTP significantly (300%) over what is seen after denervation alone (160%).

While recording the EMG response to 5-HTP in the groups

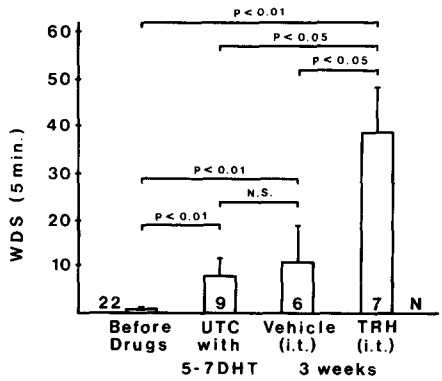


FIG. 2. Graph showing the average integrated EMG response ± SEM recorded in the extensor muscles of the thigh of spinalized rats in the thirty minutes following an injection of DL-5-HTP 100 mg/kg IP expressed in percent of the EMG activity recorded in the same animal in the thirty minutes preceding the injection. All animals were studied twenty-four to forty-eight hours after spinalization. The first group (N=10) on the left had no other treatment, the second middle bar (N=6) and the third (on the right) N=7 had received an injection of 5,7-dihydroxytryptamine intrathecally three weeks before and the third group received in addition during the three weeks preceding spinalization a continuous infusion of TRH given intrathecally. Statistical comparison was done with Student's *t*-test.

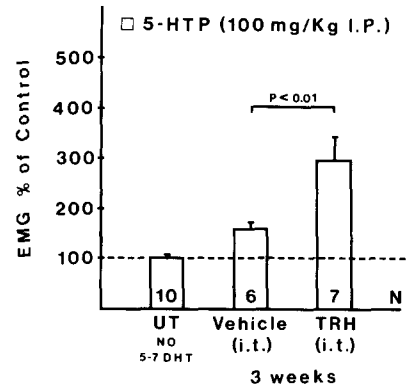


FIG. 3. Number of wet dog shakes recorded during five minutes, starting twenty minutes after an injection of DL-5-HTP 100 mg/kg IP in four groups of spinalized rats. From left to right: (a) without any other treatment; (b,c,d) three weeks after denervation with intrathecal 5,7-DHT; (c,d) with a three-week intrathecal infusion of vehicle (c) or TRH (d). Number of animals (N) is shown in the bars. Crossbars indicate SEM. Statistical analysis was done with one-way analysis of variance.

treated with TRH or vehicle, it was noted that the animals displayed numerous wet dog shakes, a phenomenon which has been shown to be related to 5-HT stimulation in forebrain structures (5).

Figure 3 shows the pattern of the wet dog shake response to 5-HTP. As compared to nondenervated animals, denervation alone with or without a three-week infusion of vehicle increases the response to 5-HTP. In such denervated animals, however, a three-week intrathecal treatment with TRH further increases the response to 5-HTP.

The rats receiving a chronic intrathecal infusion of TRH lost twenty percent of their body weight while the other groups remained stable or increased their weight slightly. Anorectic properties of TRH have been described by Vogel, Cooper, Barlow, Prange, Mueller and Breese (26).

## DISCUSSION

The present study confirms our earlier observation (2) that TRH administered systemically in paraplegic rats increases motoneuronal discharges as evidenced by increased spontaneous electromyographic activity in the hindlimbs, in a manner very similar to 5-HTP or various 5-HT agonists. This is in accordance with the observation of a direct excitatory action of TRH on spinal motoneurons (17,28). The acute effect of a single dose lasts approximately thirty minutes and does not affect the subsequent response to systemic administration of 5-HTP as opposed to substance P which also coexists with 5-HT in the descending bulbo-spinal pathway (24).

On the other hand, a chronic intrathecal infusion of TRH during the three weeks that follow the denervation with 5,7-DHT does not prevent establishment of supersensitivity as we had previously thought possible (24). On the contrary, such a chronic exposure to TRH further increases the response to systemic 5-HTP. Our experimental conditions do not allow us to pinpoint the exact site of action of TRH and the cause of this increase. Knowing however that TRH can directly excite motoneurons (17, 18, 28) and that TRH recognition sites are especially abundant in the ventral horn (19,21), we believe that TRH, administered chronically, modulates the state of responsiveness of motoneurons to other excitatory stimuli including 5-HT. This could well represent the physiological role of TRH in the descending bulbo-spinal fibers.

The modulatory action of TRH on the response to 5-HTP (or 5-HT) does not appear to be restricted to areas of the central nervous system where the amine and the peptide coexist in the same fibers such as the spinal cord (12,13). The wet dog shake phenomenon, which is seen in response to 5-HTP and 5-HT agonists, is apparently elicited in the forebrain (5) and possibly at the level of the limbic system (15) where TRH and 5-HT are present but not in the same neurons (20,23). Yet the intensity of wet dog shake response to 5-HTP was greatly enhanced by chronic treatment with TRH but not by acute treatment (data not shown). Intrathecal 5,7-DHT by itself also increased but to a lesser extent the wet dog shake response. This indicates that drugs administered intrathecally can affect structures located more rostrally in the forebrain.

In conclusion, therefore, TRH appears to modulate in a tonic manner the response of neuronal systems in both the forebrain and the ventral horn of the spinal cord to 5-HT. The coexistence of the two substances in the latter system represents some form of specialization but is not apparently essential to the interaction.

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## REFERENCES

1. Banna, N. R.; Anderson, E. G. The effect of 5-hydroxytryptamine antagonists on spinal neuronal activity. J. Pharmacol. Exp. Ther. 162:319–325; 1968.
2. Barbeau, H.; Bédard, P. J. Similar motor effects of 5-HT and TRH in rats following chronic spinal transection and 5-7 dihydroxytryptamine injection. Neuropharmacology 20:477–481; 1981.
3. Barbeau, H.; Filion, M.; Bédard, P. J. Effects of agonists and antagonists of serotonin on spontaneous hindlimb EMG activity in chronic spinal rats. Neuropharmacology 20:99–107; 1981.
4. Bédard, P. J.; Barbeau, H.; Barbeau, G.; Filion, M. Progressive increase of motor activity induced by 5-HTP in the rat below a complete section of the spinal cord. Brain Res. 169:393–397; 1979.

5. Bédard, P. J.; Pycock, C. J. *Wet Dog Shake* behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity. Neuropharmacology 16:663–670; 1977.
6. Chan-Palay, V.; Jonsson, G.; Palay, S. L. Serotonin and substance P coexist in neurons of the rat's central nervous system. Proc. Natl. Acad. Sci. USA 75:1582–1586; 1978.
7. Dahlström, A.; Fuxe, K. Evidence for the existence of monoamine neurons in the central nervous system. II Experimentally induced changes in the intraneural amine levels of bulbospinal neurons systems. Acta Physiol. Scand. 64:1–36; 1965.
8. Gibson, S. J.; Polak, J. M.; Bloom, S. R.; Wall, P. D. The distribution of nine peptides in rat spinal cord with special emphasis on the substantia gelatinosa and on the area around the central canal (lamina X). J. Comp. Neurol. 1981:65–79; 1981.
9. Gilbert, R. F. T.; Emson, P. C.; Hunt, S. P.; Bennette, G. W.; Marsden, C. A.; Sandberg, B. E. B.; Steinbusch, H. W. M.; Verhofstad, A. A. J. The effects of monoamines neurotoxins on peptides in the rat spinal cord. Neuroscience 7:69–87; 1982.
10. Hökfelt, T.; Fuxe, K.; Johansson, O.; Jeffcoate, S.; White, N. Thyrotropin releasing hormone (TRH)-containing nerve terminals in certain brain stem nuclei and in the spinal cord. Neurosci. Lett. 1:133–139; 1975.
11. Hökfelt, T.; Ljungdahl, S.; Steinbusch, H. W.; Verhofstad, A.; Nilsson, G.; Brodin, E.; Goldstein, M. Immunohistochemical evidence of substance P-like immunoreactivity in some 5-hydroxytryptamine containing neurons in the rat nervous system. Neuroscience 3: 517–538; 1978.
12. Hökfelt, T.; Lundberg, J.; Schultzberg, M.; Johansson, O.; Ljungdahl, A.; Rehfeld, J. Co-existence of peptides and putative transmitters in neurons. In: Costa, E.; Trabucchi, M., eds. Neural peptides and neural communication. New York: Raven Press; 1980:1–23.
13. Johansson, O.; Hökfelt, T.; Pernow, B.; Jeffcoate, S. L.; White, N.; Steinbusch, H. W.; Verhofsad, A. A. J.; Emson, P. C.; Spindel, E. Immunohistochemical support for three putative transmitters in one neuron: coexistence of 5-hydroxytryptamine, substance P and thyrotropin releasing hormone-like immunoreactivity in medullary neurons projecting to the spinal cord. Neuroscience 6:1857–1881; 1981.
14. Komissarov, I. V.; Abramets, I. I. Effect of monoamines on motoneurons of the isolated rat spinal cord. Translated VDC 612: 83: 612-622, 1981. Neirofiziologiya 12:391; 1980.
15. LeGal LaSalle, G.; Cavalheiro, E. A.; Feldblum, S.; Maresova, D. Studies of *Wet Dog Shake* behavior induced by septohippocampal stimulation in the rat. Can. J. Physiol. Pharmacol. 61:1299–1304; 1983.
16. Lopachin, M.; Rudy, T. A. An improved method for chronic catherization of the rat spinal subarachnoïd space. Physiol. Behav. 27:559–561; 1981.
17. Nicoll, R. A. Excitatory action of TRH on spinal motoneurons. Nature 265:242–243; 1977.
18. Ono, H.; Fukuda, H. Ventral root depolarization and spinal reflex augmentation by a TRH analog in rat spinal cord. Neuropharmacology 21:739–744; 1982.
19. Prasad, C.; Edwards, R. M. Thyrotropin-releasing hormone (TRH): apparent receptor binding in rat spinal cord. Brain Res. 311:1–6; 1984.
20. Sharif, N. A.; Burt, D. R. Limbic, hypothalamic, cortical and spinal regions are enriched in receptors for thyrotropin-releasing hormone. Evidence from [$^3$H] ultrafilm autoradiography and correlation with central effects of the tripeptides in rat brain. Neurosci. Lett. 60: 337–342; 1985.
21. Sharif, N. A.; Burt, D. R. Receptor for thyrotropin-releasing hormone (TRH) in rabbit spinal cord. Brain Res. 270:259–263; 1983.
22. Sharif, N. A.; Burt, D. R.; Towle, A. C.; Mueller, R. A.; Breese, G. R. Codepletion of serotonin and TRH induces apparent supersensitivity of spinal TRH receptors. Eur. J. Pharmacol. 95:301–304; 1983.
23. Steinbusch, H. W. M. Distribution of serotonin-immunoreactivity in the central nervous system of the rat. Neuroscience 6:557–618; 1981.
24. Tremblay, L. E.; Bédard, P. J.; Maheux, R.; DiPaolo, T. Denervation supersensitivity to 5-hydroxytryptamine in rat spinal cord is not due to the absence of 5-hydroxytryptamine. Brain Res. 330:174–177; 1985.
25. Tremblay, L. E.; Maheux, R.; Bédard, P. J. Substance P in the lumbar spinal cord of the rat affects the motor response to 5-HTP and TRH. Neuropharmacology 25:419–424; 1986.
26. Vogel, R. A.; Cooper, B. R.; Barlow, T. S.; Prange, A. J., Jr.; Mueller, R. A.; Breese, G. R. Effects of thyrotropin-releasing hormone on locomotor activity, operant performance and ingestive behavior. J. Pharmacol. Exp. Ther. 208:161–168; 1979.
27. White, S. R.; Neuman, R. S. Facilitation of spinal motoneurone excitability by 5-hydroxytryptamine and noradrenaline. Brain Res. 188:119–127; 1980.
28. White, S. R. Serotonin and co-localized peptides: Effects on spinal motoneuron excitability. Peptides 6(Suppl. 2):123–127; 1985.