J. Pharm. Pharmacol. 1984, 36: 142–144
Communicated August 4, 1983

© 1984 J. Pharm. Pharmacol.

Potentiation of cyclo(*N*-methyl-Tyr-Arg)-induced antinociceptive activity by thyrotropin-releasing hormone in mice

SHUNSUKE KAWAMURA*, SHINOBU SAKURADA, TSUKASA SAKURADA, KENSUKE KISARA, YASUYUKI AKUTSU†, YUSUKE SASAKI†, KENJI SUZUKI†, *Departments of Pharmacology and †Biochemistry, Tohoku College of Pharmacy, 4-4-1 Komatsushima, Sendai 983, Japan*

The effect of thyrotropin-releasing hormone (TRH) and its metabolite, cyclo(His-Pro) (C.HP), on cyclo(*N*-methyl-Tyr-Arg) (C.NMTA)-induced antinociception as measured by the tail-pressure test in mice has been examined. C.NMTA-induced antinociception was significantly potentiated by simultaneously intracerebroventricular or intraperitoneal injection of TRH (approximately 20–50%) in a dose-dependent manner, whereas the effect of morphine was not influenced significantly by TRH. C.HP had no significant effect on the antinociceptive response induced by C.NMTA or morphine. It is concluded that the mechanism of C.NMTA-induced antinociception may be involved in TRH neuronal system in the brain.

Thyrotropin-releasing hormone (TRH – pGlu-His-Pro-NH_2) which stimulates thyrotropin (Bowers et al 1971) and prolactin (Fleischer et al 1970) release from the anterior pituitary is isolated from the hypothalamus and has subsequently been shown to be distributed in extrahypothalamic areas of brain and gastrointestinal tract (Morley et al 1977). TRH and its metabolite, cyclo(His-Pro) (C.HP), are known to act on the central nervous system independently of their endocrine effects on the pituitary (Prange et al 1974; Prasad et al 1977). TRH or C.HP has been shown to antagonize the antinociception induced by Δ^9-tetrahydrocannabinol (THC) and neurotensin, whereas pretreatment with naloxone is without effect (Bhargava & Matwyshyn 1980; Osbahr et al 1981).

On the other hand, cyclo(*N*-methyl-Tyr-Arg) (C.NMTA), which is an analogue of kyotorphin and one of the diketopiperazine derivatives, has potent antinociceptive activity as measured by the tail-pressure test in mice (Sakurada et al 1982). Recently, we have observed that C.NMTA injected into the 3rd ventricle produced naloxone-irreversible antinociceptive activity as measured by the tail-flick test in rats (Kawamura et al 1983).

The present investigation was carried out to study the involvement of TRH and its metabolite, C.HP on C.NMTA-induced antinociception in mice.

* Correspondence.

Materials and methods

Male ddY mice, 20–24 g, were housed 22 ± 2 °C at least two days before their use for one experiment only. Food and water were freely available. A standard light-dark cycle was maintained with a timer-regulated light period from 9 a.m. to 9 p.m. All drugs for intracerebroventricular (i.c.v.) injection were freshly prepared in Ringer solution. The technique for i.c.v. injection was as described by Orikasa et al (1980). For intraperitoneal (i.p.) injection, TRH was dissolved in 0.9% NaCl (saline). The drugs used for injection were: morphine hydrochloride (Takeda); TRH (Peptide Research Foundation); C.NMTA; C.HP. The synthesis of dipeptides has been partially discussed elsewhere (Sasaki et al 1981).

Mice were evaluated for responsiveness to noxious stimuli, using the tail-pressure test which was slightly modified from the original method (Green et al 1951); the base of the tail was pressed mechanically and the level of pressure in mmHg (10 mmHg s^{-1}) that evoked biting or struggling behaviour was noted. The responsive pressure before drug injection was 43·9 ± 0·4 mmHg (N = 100). A value of 100 mmHg was used as the cut-off pressure to avoid damage of the tail. The antinociceptive activity for each mouse was calculated according to the following formula:

$$\% \text{ of antinociception} = (P_2 - P_1/100 - P_1) \times 100$$

Where P_1 is the responsive pressure before drug injection (mmHg) and P_2 is the responsive pressure after drug injection. The data are expressed as mean % of antinociceptive response ± s.e. At 5, 15, 30 and 60 min following injection, tail-pressure thresholds were redetermined. Student's *t*-test was used for comparison of several treatment groups with a control group.

Results

Table 1 shows the effect of simultaneously i.c.v. administered TRH on C.NMTA- and morphine-induced antinociceptive activity as measured by the tail-pressure test. The antinociceptive response of

Table 1. The effect of i.c.v. administered TRH on C.NMTA- and morphine (Mor)-induced antinociceptive activity in mice in the tail-pressure test. The doses (nmol/mouse) used are shown in parentheses.

		Time after injection (min)			
Treatments	n	5	15	30	60
Ringer	10	1·5 ± 3·1	1·3 ± 2·9	0·1 ± 2·9	−0·8 ± 2·0
C.NMTA (28)	20	53·2 ± 7·0†††	27·1 ± 5·7††	4·0 ± 2·6	−2·0 ± 2·2
C.NMTA (28) + TRH (2)	10	76·2 ± 8·9	49·1 ± 10·6	20·2 ± 7·5	2·7 ± 2·6
C.NMTA (28) + TRH (4)	10	82·1 ± 8·2*	61·2 ± 9·0**	28·0 ± 4·6***	3·2 ± 3·0
C.NMTA (28) + TRH (8)	10	91·5 ± 5·7**	73·2 ± 9·0***	16·4 ± 3·5*	4·8 ± 3·5
C.NMTA (28) + TRH (10)	10	94·3 ± 3·8***	77·4 ± 8·5***	45·8 ± 10·1***	8·6 ± 4·2
Mor (2)	20	38·0 ± 5·6†††	61·7 ± 6·7†††	49·9 ± 4·7†††	23·5 ± 3·6†††
Mor (2) + TRH (2)	10	51·0 ± 9·4	70·1 ± 10·1	65·6 ± 8·6	31·0 ± 4·8
Mor (2) + TRH (4)	10	59·7 ± 9·9‡	67·5 ± 8·6	62·3 ± 7·4	31·5 ± 3·7
Mor (2) + TRH (8)	10	69·1 ± 10·6‡‡	68·1 ± 8·0	50·1 ± 12·2	21·2 ± 10·9
Mor (2) + TRH (16)	10	69·8 ± 9·1‡‡	71·9 ± 7·0	61·2 ± 5·5	33·8 ± 4·7
TRH (2)	20	11·1 ± 4·0	1·2 ± 2·6	−3·8 ± 1·5	−3·9 ± 1·7
TRH (4)	20	18·8 ± 3·3††	2·2 ± 2·1	−3·7 ± 2·3	−4·8 ± 2·1
TRH (8)	20	18·5 ± 3·6††	−0·1 ± 2·5	−4·1 ± 2·6	−4·5 ± 1·7
TRH (16)	20	23·3 ± 5·0††	4·8 ± 3·8	−3·8 ± 3·5	−7·7 ± 3·1

Each value was expressed as % of antinociception.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ when compared with C.NMTA (28 nmol/mouse) alone.
‡$P < 0.05$, ‡‡$P < 0.01$ when compared with morphine (2 nmol/mouse) alone.
††$P < 0.01$, †††$P < 0.001$ when compared with Ringer solution.

C.NMTA was significantly potentiated by simultaneous injection of TRH (4, 8 and 16 nmol/mouse, i.c.v.) in a dose-dependent manner. This potentiating effect lasted for 30 min post-injection. The inhibition of the tail-pressure response induced by morphine and TRH (4, 8 and 16 nmol/mouse, i.c.v.) was observed in an additive manner at 5 min post-injection. TRH alone had a significant ($P < 0.01$) antinociceptive activity of approximately 11 to 23% at 5 min post-injection compared with the Ringer control. Although the antinociceptive activity of TRH disappeared 15 min after injection, C.NMTA-induced antinociception was significantly potentiated by TRH (approximately 20–50%), whereas the effect of morphine was not influenced significantly by TRH. Likewise, the antinociceptive activity of i.c.v. injected C.NMTA immediately after peripheral injection of TRH (2·5, 5 and 10 mg kg^{-1} i.p.) was significantly potentiated by TRH (approximately 20–45%) for 30 min post-injection (Table 2).

Table 3 shows the effect of simultaneously i.c.v. administered C.HP, which is known to be an active metabolite of TRH, or one of diketopiperazine derivatives, on C.NMTA- and morphine-induced antinociceptive activity as measured by the tail-pressure test in mice. C.HP (2, 4, 8 and 16 nmol/mouse, i.c.v.) had no significant effect on the antinociceptive response induced by C.NMTA or morphine.

Discussion

The findings clearly indicate C.NMTA-induced antinociception to be significantly potentiated by i.c.v. or i.p. injection of TRH which itself produced a slight effect. Though the half life of TRH is short (Redding & Schally 1972), TRH potentiated C.NMTA-induced

Table 2. The effect of i.p. administered TRH on C.NMTA-induced antinociceptive activity in mice in the tail-pressure test. The doses of TRH (mg kg^{-1}) and C.NMTA (nmol/mouse, i.c.v.) are shown in parentheses.

		Time after injection (min)			
Treatment	n	5	15	30	60
Ringer + saline	10	2·1 ± 2·1	0·8 ± 1·7	1·1 ± 1·6	2·1 ± 2·4
C.NMTA (28 + saline	20	51·0 ± 5·1†††	25·2 ± 3·8†††	−1·1 ± 2·0	−2·7 ± 2·4
C.NMTA (28)+TRH (2·5)	10	56·1 ± 6·4	34·0 ± 7·1	20·7 ± 5·0***	0·3 ± 2·5
C.NMTA (28) + TRH (5)	10	68·5 ± 8·0	48·7 ± 4·3***	20·9 ± 4·8***	3·6 ± 2·5
C.NMTA (28) + TRH (10)	10	92·2 ± 4·9***	70·6 ± 8·1***	36·8 ± 4·5***	6·8 ± 3·0*
Ringer + TRH (2·5)	10	3·3 ± 2·5	1·1 ± 2·3	0·8 ± 1·6	−1·5−2·0
Ringer + TRH (5)	10	7·9 ± 3·1	−0·4 ± 2·2	−1·1 ± 1·4	−1·9 ± 2·1
Ringer + TRH (10)	10	12·2 ± 2·9*	3·9 ± 2·0	−0·3 ± 2·5	−2·5 ± 1·3

Each value was expressed as % of antinociception.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ when compared with C.NMTA (28) plus saline control.
†$P < 0.05$, †††$P < 0.001$ when compared with Ringer plus saline control.

Table 3. The effect of i.c.v. administered C.HP on C.NMTA- and morphine (Mor)-induced antinociceptive activity in mice in the tail-pressure test. The doses (nmol/mouse) used are shown in parentheses.

		Time after injection (min)			
Treatments	n	5	15	30	60
Ringer	10	1·5 ± 3·1	1·3 ± 2·9	0·1 ± 2·9	− 0·8 ± 2·0
C.NMTA (28)	20	53·2 ± 7·0†††	27·1 ± 5·7††	4·0 ± 2·6	− 2·0 ± 2·2
C.NMTA (28) + C.HP (2)	10	55·4 ± 8·5	30·5 ± 7·1	2·0 ± 3·1	− 1·3 ± 2·6
C.NMTA (28) + C.HP (4)	10	55·0 ± 7·6	32·2 ± 6·6	2·9 ± 2·0	− 0·3 ± 1·8
C.NMTA (28) + C.HP (8)	10	56·4 ± 5·9	32·4 ± 6·1	3·3 ± 1·8	− 0·8 ± 1·4
C.NMTA (28) + C.HP (16)	10	55·8 ± 8·1	30·6 ± 5·9	2·7 ± 3·2	− 0·2 ± 1·4
Mor (2)	20	34·7 ± 5·3†††	59·3 ± 9·0†††	48·0 ± 6·1†††	25·1 ± 6·2†††
Mor (2) + C.HP (2)	10	39·6 ± 3·7	54·0 ± 7·6	41·6 ± 6·4	20·5 ± 5·0
Mor (2) + C.HP (4)	10	34·4 ± 5·0	54·6 ± 7·5	45·5 ± 5·3	21·5 ± 3·0
Mor (2) + C.HP (8)	10	40·3 ± 4·9	53·0 ± 7·1	42·1 ± 5·3	22·7 ± 3·8
Mor (2) + C.HP (16)	10	40·2 ± 4·1	55·5 ± 6·6	38·0 ± 5·2	29·2 ± 5·4
C.HP (2)	10	3·8 ± 2·3	0·2 ± 2·8	1·8 ± 1·7	0·4 ± 2·2
C.HP (4)	10	2·3 ± 1·9	1·7 ± 2·0	− 0·6 ± 1·8	0·6 ± 2·0
C.HP (8)	10	− 0·9 ± 1·9	− 1·1 ± 2·6	− 2·2 ± 2·3	− 1·8 ± 2·7
C.HP (16)	10	5·8 ± 1·3	0·7 ± 2·0	3·1 ± 1·1	− 1·1 ± 2·2

Each value was expressed as % of antinociception.
††$P < 0·01$, †††$P < 0·001$ when compared with Ringer solution.

antinociception even at 30 min post-injection. On the other hand, C.NMTA was unaffected by C.HP which is known as an active metabolite of TRH. It is, therefore, inferred that potentiation of C.NMTA-induced antinociception by TRH may not be mediated by TRH conversion to an active metabolite C.HP in the brain. The inhibitory response of morphine on mechanically applied noxious stimuli was slightly increased by simultaneous treatment with TRH at 5 min post-injection. This increased threshold must be raised by TRH, since TRH itself produced a weak antinociceptive effect. From these results morphine appears to have acted in an additive manner in contrast to C.NMTA. Moreover, the results indicate that the mode of antinociceptive activity of C.NMTA is different from that of morphine.

It has been reported that the antinociceptive response of THC which is not reversed by naloxone, an opiate antagonist, is antagonized by TRH and C.HP (Bhargava & Matwyshyn 1980). Subsequently, TRH administered centrally and peripherally antagonized neurotensin-induced non-narcotic antinociceptive activity in three analgesic tests (Osbahr et al 1981). Sakurada et al (1983) have already reported that C.NMTA has potent antinociceptive activity which is incompletely reversed by naloxone (2 mg kg^{-1} i.p.) in mice in three antinociceptive tests. In the present experiment, C.NMTA-induced antinociception was not reversed, but potentiated, by TRH. It is therefore suggested that the features of C.NMTA-induced antinociception may be different from those of THC or neurotensin. It also seems that C.NMTA may have a unique central mechanism of antinociception unlike opioid analgesics.

In summary, C.NMTA has been demonstrated to produce a much more TRH-potentiated antinociception than morphine. It is concluded that the mechanism of C.NMTA-induced antinociception may be involved in TRH neuronal system in the brain.

This work has been supported by a research grant No 57570081 from the Japanese Ministry of Education, Science and Culture.

REFERENCES

Bhargava, H. N., Matwyshyn, G. A. (1980) Eur. J. Pharmacol. 68: 147–154

Bowers, C. Y., Friesen, H. G., Hwang, P., Guyda, H. J., Folkers, K. (1971) Biochem. Biophys. Res. Commun. 45: 1033–1041

Fleischer, N., Burgus, R., Vale, W., Dunn, T., Guillemin, R. (1970) J. Clin. Endocrinol. Metab. 31: 109–112

Green, F., Young, D. A., Goldfrey, E. I. (1951) Br. J. Pharmacol. 6: 572–585

Kawamura, S., Sakurada, S., Sakurada, T., Kisara, K., Akutsu, Y., Sasaki, Y., Suzuki, K. (1983) Eur. J. Pharmacol. 93: 1–8

Morley, J. E., Levine, A. S., Prasad, C. (1977) Brain Res. 210: 475–478

Orikasa, S., Sakurada, S., Kisara, K. (1980) Psychopharmacol. 67: 53–59

Osbahr, A. J., Nemeroff, C. B., Luttinger, D., Mason, G. A., Prange, A. J. (1981) J. Pharmacol. Exp. Ther. 217: 645–651

Prange, A. J., Breese, G. R., Cott, J. M., Martin, B. R., Copper, B. R., Wilson, I. C., Plotnikoff, N. P. (1974) Life Sci. 14: 447–455

Prasad, C., Matsui, T., Peterkofsky, A. (1977) Nature (London) 268: 3229–3234

Redding, T. W., Schally, A. V. (1972) Neuroendocrinology 9: 250–256

Sakurada, S., Sakurada, T., Jin, H., Sato, T., Kisara, K., Sasaki, Y., Suzuki, K. (1982) J. Pharm. Pharmacol. 34: 750–751

Sakurada, T., Sakurada, S., Watanabe, S., Kawamura, S., Sato, T., Kisara, K., Akutsu, Y., Sasaki, Y., Suzuki, K. (1983) Neuropharmacol. in the press

Sasaki, Y., Akutsu, Y., Matsui, M., Suzuki, K., Sakurada, S., Sato, T., Kisara, K. (1982) Chem. Pharm. Bull. 30: 4435–4443