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Antinociceptive activities of synthetic dipeptides in mice

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A dipeptide, kyotorphin (L-Tyrosyl-L-Arginine) was isolated from bovine brain by Takagi et al (1979a) who observed that administered intracisternally to mice, this peptide had an antinociceptive action which was about 4.2 times more potent than met-enkephalin, and which was abolished by pretreatment with naloxone. Kyotorphin had no inhibitory effect on electrically-induced contractions of longitudinal muscle of guinea-pig ileum (Takagi et al 1979b). The peptide is also present in the midbrain, the pons plus medulla oblongata and the dorsal horn of spinal cord where its distribution differs slightly from that of met-enkephalin (Ueda et al 1980).

The present study was undertaken to investigate antinociceptive effects of cyclic dipeptides which are kyotorphin analogues or diketopiperazine derivatives in order to obtain dipeptides with much longer duration and more potent antinociceptive action than kyotorphin or met-enkephalin.

Method

Antinociceptive activity was assessed in adult male dd-Y mice (18–20 g) using the tail pressure test (Takagi et al 1957) which was slightly modified from the original method (Green et al 1951). The base of the tail was pressed and the level of pressure in mm Hg (10 mm Hg s^{-1}) that evoked biting or struggling behaviour was noted. Groups of mice (n = 10) were selected for each experiment, no animals being used more than once. Only mice responding behaviourally to the mechanical nociceptive stimulation (40–50 mm Hg) were selected. Following determination of pre-drug values, animals were injected with dipeptides dissolved with Ringer solution (NaCl 8·6 g, KCl 0·3 g and CaCl 0·33 g, distilled water 1000 ml). The technique for intracerebroventricular injection (i.c.v.) was described previously (Orikasa et al 1980). At 5, 15, 30, 45 and 60 min following injection, tail pressure thresholds were redetermined except for met-enkephalin. Antinociceptive activity was determined to be positive when the tail pressure was increased over 60–75 mm Hg and ED50 values were determined by the method of Lichfield & Wilcoxon (1949). All drugs were dissolved in Ringer solution. The synthesis of dipeptides has been partially discussed elsewhere (Sasaki et al 1981).

Results

Table 1 shows the structure of cyclic dipeptides and the relative potency of compounds compared with met-enkephalin. The peak antinociceptive activity for met-enkephalin was observed at 2 min with a falling away at 10 min. The injection of cyclo(L-Tyr-L-Arg) into the lateral cerebroventricle produced a five-fold increase in antinociceptive potency compared with met-enkephalin. Synthetic kyotorphin (L-Tyr-L-Arg) was less active than met-enkephalin, whereas the stereoisomer of kyotorphin enhanced potency to about six times that of kyotorphin and to more than twice that of met-enkephalin. On the other hand, the aminomethylation of cyclo(L-Tyr-L-Arg) produced the most potent dipeptide, cyclo(*N*-methyl-L-Tyr-L-Arg), of all the compounds investigated which was about 7 times more potent than met-enkephalin (Table 2). Aminomethylation also enhanced the potency of cyclo(L-Tyr-D-Arg). Cyclo(L-Tyr-L-His) and cyclo(L-Tyr-L-Lys) had no marked effects, whereas substitution of L-phenylalanine and L-tryptophan for L-tyrosine in the structure of cyclo (L-Tyr-L-Arg) resulted in a compound with potent antinociceptive activity approximately 4 times as active as met-enkephalin. All dipeptides gave a peak antinociceptive activity at 5 min falling away at 60 min. To confirm the morphine-like specificity of some of these derivatives, antagonism by naloxone was tested. Antinociceptive activity produced by 250 nmol/mouse (ED100 dose) of met-enkephalin was dramatically antagonized by intraperitoneal injection (i.p.) of naloxone (2 mg kg^{-1}) given 10 min before to i.c.v. injection of met-enkephalin. However, the antinociceptive effect of cyclo(*N*-methyl-L-Tyr-L-Arg), the most active of the peptides, was antagonized incompletely (approximately 40%) by naloxone (2 mg kg^{-1} i.p.).

Discussion

The data show that cyclic dipeptides can be modified to give compounds highly active in the mouse tail pressure test. A cyclic ring is therefore one way of increasing potency but this does not exclude untried structural changes. The enhanced potency of cyclic dipeptides, relative to linear dipeptides, is theorized to arise from increased resistance to enzymatic destruction. Cyclo(*N*-methyl-L-Tyr-L-Arg) was the most potent cyclic dipeptide in the present study and the ED50 value was 23·5 nmol/animal. Furthermore, it is apparent that definite naloxone-reversible antinociceptive efficacy was not observed after i.c.v. injection of cyclo(*N*-methyl-L-Tyr-L-Arg) in contrast with met-enkephalin which was completely blocked by naloxone.

Our data are in line with the previous data (Takagi et

† Correspondence.

Table 1. Antinociceptive activities produced by i.c.v. administration in the mouse tail pressure test. Relative potency is on a molar basis (met-enkephalin = 1·00)

Compounds	n	Peak time (min)	ED50* (nmol/animal)	Relative potency
Met-enkephalin	30	2	170·0 (146·6–197·2)	1·00
L-Tyr-L-Arg (Kyotorphin)	30	5	393·0 (342·3–452·7)	0·43
L-Tyr-D-Arg	30	5	71·0 (59·6– 84·4)	2·39
Cyclo(L-Tyr-L-Arg)	30	5	33·0 (26·9– 40·4)	5·15
Cyclo(L-Tyr-D-Arg)	30	5	435·0 (233·2–587·2)	0·39
Cyclo(D-Tyr-L-Arg)	30	5	250·0 (172·4–362·5)	0·68
Cyclo(D-Tyr-D-Arg)	30	5	85·0 (48·6–148·8)	2·00
Cyclo(*N*-methyl-L-Tyr-L-Arg)	30	5	23·4 (15·5– 35·5)	7·26
Cyclo(*N*-methyl-L-Tyr-D-Arg)	30	5	125·7 (68·9–235·8)	1·35
Cyclo(*N,O*-dimethyl-L-Tyr-L-Arg)	30	5	58·8 (40·3– 85·9)	2·89
Cyclo(L-Tyr-L-His)	30	5	260·0 (170·4–396·5)	0·65
Cyclo(L-Tyr-L-Lys)	30	5	103·0 (74·9–141·6)	1·65
Cyclo(L-His-L-Pro)	30	5	507·0 (316·8–811·2)	0·34
Cyclo(L-Phe-L-Arg)	30	5	47·1 (32·0– 69·2)	3·61
Cyclo(L-Trp-L-Arg)	30	5	41·0 (31·2– 52·1)	4·15

* ED50 values were calculated from the values obtained at the time of peak effect.
95% confidence limits are given in parentheses.

Table 2. The time-course effect of cyclo (*N*-methyl-L-Try-L-Arg) and met-enkephalin in the mouse tail pressure method.

	Time after injection						
Peptides	2 min	5 min	10 min	15 min	30 min	45 min	60 min
Ringer solution	45·0 ± 2·1	46·1 ± 1·7	46·2 ± 1·8	47·0 ± 2·0	49·1 ± 2·1	48·8 ± 1·7	47·5 ± 1·3
Cyclo(*N*-methyl-L-Tyr-L-Arg) 28nmol/mouse	—	83·4 + 6·5**	—	—	64·8 + 2·4*	56·2 + 1·9*	52·4 + 2·9
Met-enkephalin 250 nmol/mouse	91·9 ± 4·3**	88·7 ± 3·5**	49·9 ± 3·1	45·7 ± 1·3	—	—	—

Each value was expressed as mm Hg.
* $P < 0·01$ when compared with Ringer control.
** $P < 0·001$ when compared with Ringer control.

al 1979b) that replacement of the L-arginine moiety of kyotorphin by D-arginine, produced a significant elevation in the potency of antinociceptive activity to about 5·5 times compared with kyotorphin. However, it is highly probable that the difference in the ED50 value between two experiments may be due to the difference in injection sites between the present study compared with those of Takagi et al (1979b). The inhibition of the tail pressure response induced by cyclo(*N*-methyl-L-Tyr-L-Arg) was not completely reversed by 2 mg kg^{-1} i.p. of naloxone. This phenomenon agrees with the recent report that the tail flick inhibition induced by the intrathecal administration of D-Ala^2-D-Leu^5-Enk was not reversed by 2 mg kg^{-1} of naloxone and was incompletely antagonized by the higher dose of 6 mg kg^{-1} of naloxone (Tseng 1981). It may be that only part of the action is via an opiate receptor interaction and the residual activity is via a completely different non-opiate mechanism.

From the present experiment, it is concluded that the addition of a cyclic ring to the chemical structure of the linear dipeptides, kyotorphin and analogues gives active cyclic dipeptide analogues as assessed in the mouse tail pressure test. The results also indicate that *N*-methylation of the L-tyrosine residue of cyclo(L-Tyr-L-Arg) dramatically increased the antinociceptive activity which was incompletely reversible by naloxone.

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