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Antinociceptive Cross-Tolerance Between [D-Arg^2]-Dermorphin Tetrapeptide Analogs and Morphine

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CHAKI, K , S SAKURADA, T. SAKURADA, N NAKATA, K KISARA, H WATANABE AND K SUZUKI. *Antinociceptive cross-tolerance between [D-Arg^2]-dermorphin tetrapeptide analogs and morphine* PEPTIDES **11**(1) 139–144, 1990 —Cross-tolerance between [D-Arg^2]-dermorphin tetrapeptide analogs and morphine with respect to antinociception was examined in the present set of experiments Systemic administration of H-Tyr-D-Arg-Phe-Gly-NH_2 (TDAPG-NH_2), H-Tyr-D-Arg-Phe-β-Ala-OH (TDAPA) or morphine over a period of 5 days produced the development of tolerance. In the cross-tolerance study, antinociception after subcutaneous (SC), intracerebroventricular (ICV) and intrathecal (IT) administrations of TDAPG-NH_2 and TDAPA in morphine-tolerant mice was not significantly different from their respective effects in saline-pretreated control mice A marked tolerance to SC- and ICV-administered morphine was seen in mice made tolerant to TDAPG-NH_2 and TDAPA However, IT administration of morphine produced no significant decrement in the antinociceptive activity in mice made tolerant to TDAPG-NH_2 and TDAPA These data indicate that [D-Arg^2]-dermorphin tetrapeptide analogs can produce significant antinociception in morphine-tolerant mice

Antinociception Cross-tolerance [D-Arg^2]-dermorphin tetrapeptide analogs Morphine

AN amphibian skin peptide, dermorphin, possesses a powerful morphine-like opioid activity in laboratory animals and isolated preparations (2, 3, 11–13) The presence of a D-Ala^2 moiety in its amino acid sequence and the N-terminal tetrapeptide is of crucial importance for opioid activity of dermorphin (2, 8, 23). Sato *et al.* studied the opioid activities of D-Arg^2 substituted N-terminal tetrapeptide of dermorphin and its analogs, and showed that these analogs are more powerful than dermorphin tetrapeptide with a D-Ala^2 residue in several assays (24).

Tolerance and physical dependence are common consequences of chronic administration of the opioids. However, the mechanisms of tolerance and dependence are uncertain. Our previous and recent experiments showed that the intensity of physical dependence, using the decrease of body weight and naloxone-precipitated withdrawal syndrome as indices, was much weaker with [D-Arg^2]-dermorphin tetrapeptide analogs than with morphine (4,19).

The present experiments were carried out to estimate the degree of tolerance to the antinociceptive effects produced by repeated systemic administrations of [D-Arg^2]-dermorphin tetrapeptide analogs as compared to morphine Subsequently, additional experiments were performed to see characteristics of cross-tolerance between [D-Arg^2]-dermorphin tetrapeptide analogs and morphine with respect to the antinociception.

METHOD

Animals

Male mice (Std-ddY strain) weighing 20–25 g were used in all experiments. The animals were housed at 22 ± 2°C, with free access to food and water. A standard light-dark cycle was maintained with a time-regulated light period from 9 00 a.m. to 9·00 p.m. Groups of 10 mice were used for each experiment

Drugs and Administration Procedures

[D-Arg^2]-dermorphin tetrapeptide analogs, H-Tyr-D-Arg-Phe-Gly-NH_2 (TDAPG-NH_2) and H-Tyr-D-Arg-Phe-β-Ala-OH (TDAPA) were synthesized by the conventional liquid phase method Morphine hydrochloride was obtained from Sankyo. Each compound was dissolved in physiological saline and administered SC in each volume of 0.1 ml/10 g of body weight. The compounds for ICV and IT administrations were dissolved in sterile Ringer's solution The final pH of all compounds for ICV and IT administrations was approximately 6.5. For ICV administration, a slightly modified method of Haley and McCormick (14) was used, with a constant volume of 10 μl/mouse. IT administration procedure was adapted from the method of Hylden and Wilcox (15) with a constant volume of 5 μl/mouse. A 29-gauge needle connected to a Hamilton microsyringe was directly inserted between the L5 and L6 segments in mice in a rate of 5 μl/10 sec.

Antinociceptive Assay

The antinociceptive activity of each compound was assessed using the tail pressure method as previously described (22). Briefly, mechanical pressure was applied to the base of the tail at

a rate of 10 mmHg/sec and biting or struggling behavior in mice to which pressure was applied mechanically was an indication of response threshold Only mice responding behaviorally to a tail pressure of 40 to 50 mmHg were selected for this assay. To avoid the tail tissue damage, a cut off of 100 mmHg was imposed on animals failing to bite or struggle The antinociceptive activity for each animal was calculated according to the following formula and expressed as % of maximum possible effect (% of MPE); % of MPE = $(P_2 - P_1/100 - P_1) \times 100$, where P_1 and P_2 are predrug responsive pressure (mmHg) and postdrug responsive pressure (mmHg), respectively

Tolerance Development of [D-Arg^2]-Dermorphin Tetrapeptide Analogs and Morphine

The AD_{90} dose (90% of MPE) of each compound to inhibit the tail pressure assay in naive mice was employed for systemic administration. The AD_{90} doses of TDAPG-NH_2, TDAPA and morphine were 2.0, 1.25 and 14.0 mg/kg, respectively This dose of each compound was administered SC once daily for 5 days. The antinociceptive activity was measured at the peak time of each compound (morphine 30 min, both peptides 45 min) obtained from the tail pressure assay in naive mice. Control groups were treated with saline solution during a period of 5 days

Cross-Tolerance Between [D-Arg^2]-Dermorphin Tetrapeptide Analogs and Morphine

On the 6th day, 2 0 mg/kg of TDAPG-NH_2 or 1.25 mg/kg of TDAPA was administered SC in mice made tolerant to morphine The antinociceptive effect was measured at 45 min after TDAPG-NH_2 or TDAPA administration. Morphine (14 mg/kg) was administered SC in mice made tolerant to TDAPG-NH_2 and TDAPA and measured at 30 min postinjection

The AD_{90} dose of each compound obtained from the previous tail pressure assay in mice (5) was employed for ICV or IT administration.

On the 6th day, 15 0 pmol of TDAPG-NH_2 or 6.8 pmol of TDAPA was administered ICV in mice made tolerant to morphine, and 5300 pmol of morphine was administered ICV in mice made tolerant to TDAPG-NH_2 and TDAPA. The antinociceptive activity was measured at 10 min after TDAPG-NH_2, TDAPA or morphine administration.

On the 6th day, the AD_{90} dose of the peptide (3 5 pmol of TDAPG-NH_2, 2.3 pmol of TDAPA) was given into the spinal subarachnoid space (IT) in mice made tolerant to morphine The AD_{90} dose of morphine (2200 pmol) was administered IT in mice made tolerant to TDAPG-NH_2 and TDAPA. The antinociceptive activity was measured at 10 min postinjection.

On the day of cross-tolerance study, AD_{90} dose of each compound was also injected into the mice treated with saline chronically and the antinociceptive activity was examined as a control

Statistics

The AD_{50} (antinociceptive dose = 50% of MPE) values and their 95% confidence limits were determined by the method of Litchfield and Wilcoxon (17). These values were calculated from the values obtained at the time of peak effect after TDAPG-NH_2, TDAPA or morphine administration. Statistical significance of the data was estimated by an analysis of variance (ANOVA) with Dunnett's test

RESULTS

Inhibition of the Tail Pressure Response to TDAPG-NH_2, TDAPA and Morphine in Naive Mice

As presented in Fig 1, SC administration of various doses of TDAPG-NH_2, TDAPA or morphine resulted in a dose-dependent antinociceptive activity TDAPG-NH_2 and TDAPA were 6 4 and 8 5 times more potent than morphine in inhibiting the tail pressure response (Table 1) The antinociceptive activity produced by TDAPG-NH_2 or TDAPA was of longer duration than that induced by morphine, with peak effect not seen until 45 min and total duration of over 240 min postinjection. In contrast, a maximally effective dose of morphine peaked at 30 min, and the activity completely disappeared 180 min after SC administration (data not shown)

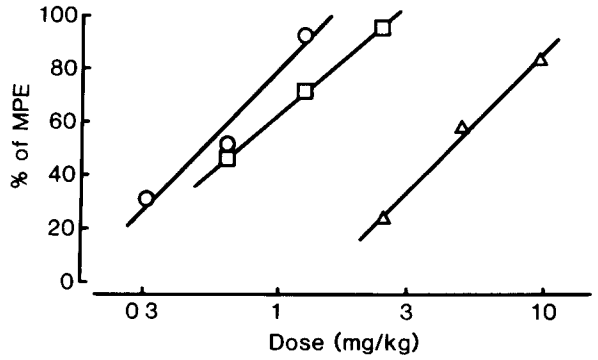

FIG 1 The antinociceptive activity produced by SC administration of various doses of TDAPG-NH_2 (□, 0 625, 1 25 and 2 5 mg/kg), TDAPA (○, 0 3125, 0 625 and 1 25 mg/kg) and morphine (△, 2 5, 5, 10 mg/kg) as measured by the tail pressure test in mice Each point represents the mean of 10 mice

Tolerance Induced by Repeated Systemic Administration of TDAPG-NH_2, TDAPA or Morphine

The time course of changes in tail pressure threshold during the repeated systemic administration of TDAPG-NH_2, TDAPA or morphine is shown in Figs 2 and 3. Morphine produced a progressive decline in nociception during 5 successive administrations. In the degree of tolerance, morphine was more pronounced than TDAPG-NH_2 or TDAPA. In morphine-treated groups, the MPE values were only 35–40% on the 5th day, whereas MPE values were 50–55% in mice treated with TDAPG-NH_2 or TDAPA.

Cross-Tolerance

The SC administration of TDAPG-NH_2 or TDAPA on day 6 significantly enhanced antinociceptive activity in morphine-tol-

TABLE 1

ANTINOCICEPTIVE ACTIVITY PRODUCED BY SC ADMINISTRATION OF EACH COMPOUND AS MEASURED BY THE TAIL PRESSURE TEST IN MICE

Compounds	AD_{50} (mg/kg)	Relative Potency	Peak Time (min)
TDAPG-NH_2	0 72 (0 44–1 19)	6 4	45
TDAPA	0 54 (0 35–0 83)	8 5	45
Morphine	4 60 (2 99–7 07)	1 0	30

AD_{50} values were calculated from the values obtained at the time of peak effect Values found in parentheses are 95% confidence limits

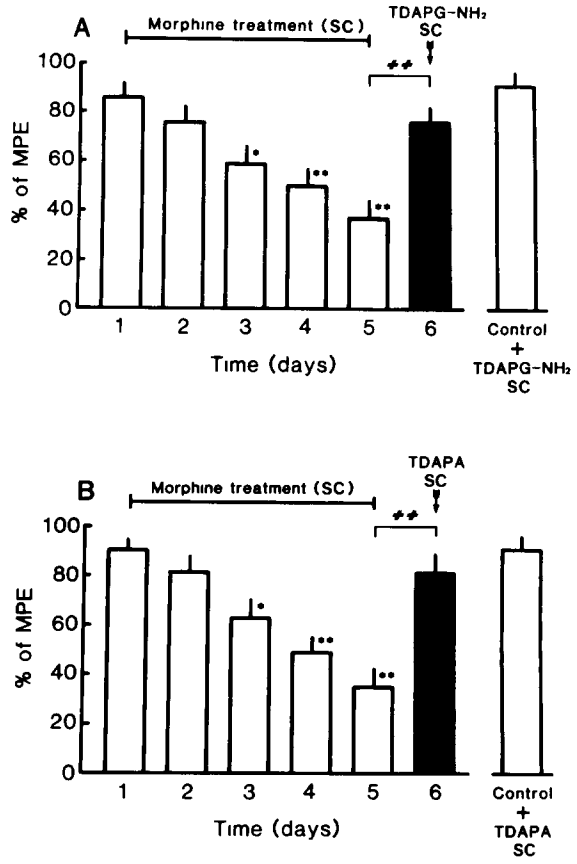

FIG 2 The time course of the development of tolerance to SC-administered morphine (14 mg/kg) (A,B) On the 6th day, morphine-tolerant mice were treated with TDAPG-NH_2 (2 0 mg/kg, SC) (A) and TDAPA (1 25 mg/kg, SC) (B), respectively Similarly, saline-pretreated (control) mice were treated with TDAPG-NH_2 (2 0 mg/kg, SC) (A) and TDAPA (1 25 mg/kg, SC) (B), respectively Each column represents the mean, with vertical lines showing s e.m of 10 mice. Significant differences from the 1st day of morphine treatment are indicated by $*p<0$ 05, $**p<0.01$ Significant differences between the 5th day following successive administrations of morphine and SC peptide in morphine-tolerant mice are indicated by $\#\#p<0.01$

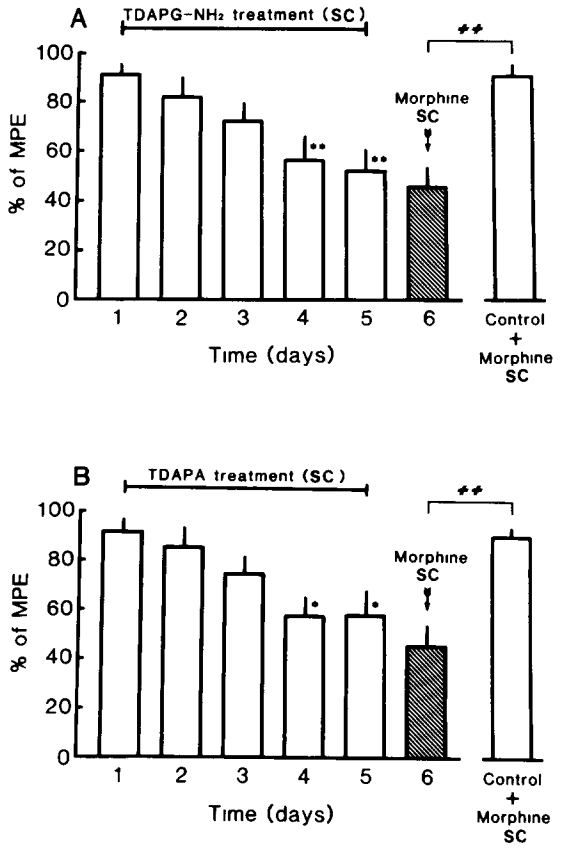

FIG 3 The time course of the development of tolerance to SC-administered TDAPG-NH_2 (2 0 mg/kg) (A) and TDAPA (1 25 mg/kg) (B) On the 6th day, all peptide-tolerant mice were treated with morphine (14 mg/kg, SC) (A,B) Similarly, saline-pretreated (control) mice were treated with morphine (14 mg/kg, SC) (A,B). Significant differences from the 1st day of all peptide treatment are indicated by $*p<0.05$, $**p<0$ 01 Significant differences between SC morphine in peptide-tolerant mice and SC morphine in control mice are indicated by $\#\#p<0$ 01 For other details see Fig 2

erant mice, as compared to the level of antinociception observed on day 5 of acute morphine administration (Fig. 2). The MPE values for TDAPG-NH_2 and TDAPA were 75.9 and 81.9%, respectively, on the day of cross-tolerance experiment in morphine-tolerant mice. These values were not significantly different from their respective values in saline-pretreated control mice. In contrast, tolerance to SC administration of morphine was observed in mice made tolerant to TDAPG-NH_2 or TDAPA (Fig. 3). The MPE values for morphine in TDAPG-NH_2- and TDAPA-tolerant mice were 46.1 and 45.5%, respectively, on the day of cross-tolerance experiment, which were significantly different from the value (91.1%) obtained in saline-pretreated control mice.

Figure 4 indicated that ICV-administered TDAPG-NH_2 or TDAPA in morphine-tolerant mice resulted in a significant enhancement of the antinociceptive activity, as compared to the level of antinociception observed on day 5 of acute morphine administration. The MPE values for ICV-administered TDAPG-NH_2 and TDAPA in morphine-tolerant mice were 65.1 and 67.1%, respectively. They were not significantly different from their respective values in saline-pretreated control mice. Conversely, Fig. 5 indicated that a marked tolerance to ICV administration of morphine was observed in mice made tolerant to TDAPG-NH_2 and TDAPA. The MPE values for ICV-administered morphine in TDAPG-NH_2- and TDAPA-tolerant mice were 36.6 and 36.1%, respectively. In saline-pretreated control mice, the MPE values for TDAPG-NH_2, TDAPA and morphine by ICV route were 85.6, 84.2 and 89.8%, respectively.

IT administration of TDAPG-NH_2 or TDAPA in morphine-tolerant mice resulted in a significant enhancement of the antinociceptive activity on the 6th day as compared with the activity on the 5th day (Fig. 6). The MPE values obtained by IT-administered TDAPG-NH_2 and TDAPA in morphine-tolerant mice were 76.2 and 74.0%, respectively. Similarly, no significant tolerance to IT administration of morphine was observed in mice made tolerant to TDAPG-NH_2 and TDAPA (Fig. 7) The MPE values for IT-administered morphine in TDAPG-NH_2 and TDAPA were 72.2 and 68.9%, respectively From their MPE values, antinociception produced by IT administration of TDAPG-NH_2 or TDAPA in morphine-tolerant mice, and IT administration of morphine in TDAPG-NH_2- or TDAPA-tolerant mice were not significantly different from their respective effects in saline-pretreated control mice. The MPE values for IT administrations of TDAPG-NH_2, TDAPA and morphine in saline-pretreated control mice were

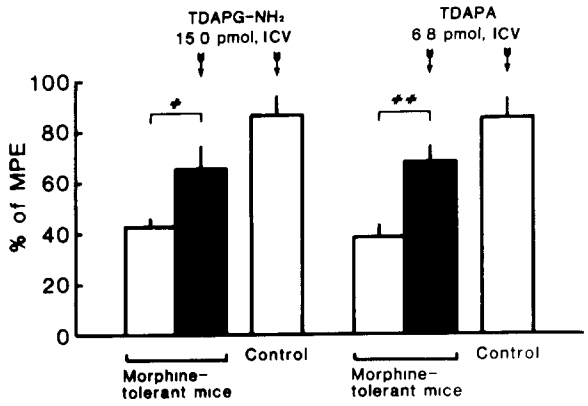

FIG 4 Cross-tolerance to ICV-administered TDAPG-NH_2 and TDAPA in mice made tolerant to morphine Each column represents the mean, with vertical lines showing s e m of 10 mice Each left open column in morphine-tolerant mice indicates the value on the 5th day following successive administrations of morphine Significant differences between the 5th day following successive administrations of morphine and ICV peptide in morphine-tolerant mice are indicated by #$p<0$ 05, ##$p<0$ 01

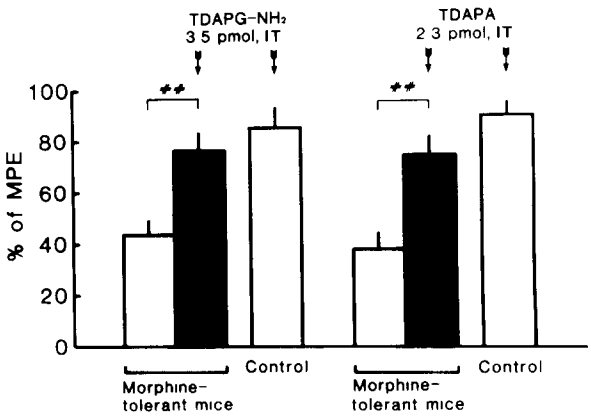

FIG 6 Cross-tolerance to IT-administered TDAPG-NH_2 and TDAPA in mice made tolerant to morphine Each open column in morphine-tolerant mice indicates the value on the 5th day following successive administrations of morphine Significant differences between the 5th day following successive administrations of morphine and IT peptide in morphine-tolerant mice are indicated by ##$p<0$ 01 For other details see Fig 4

84 7, 89.1 and 85 9%, respectively.

DISCUSSION

The phenomenon of cross-tolerance to opioid drugs with respect to antinociception involves numerous factors A previous study in our laboratory indicated that peripherally administered [D-Arg^2, Sar^4]-dermorphin tetrapeptide (H-Tyr-D-Arg-Phe-Sar-OH) was active in spinalized rats as well as rats with an intact spinal cord (unpublished data). Our recent experiment showed that the antinociceptive activity produced by the direct lumbar administration of TDAPG-NH_2 and TDAPA was approximately 6 times more potent than the activity induced by ICV administration of each peptide (5) Conversely, Wood *et al* investigated the analgesic effect of morphine in the spinalized rats and strongly suggest that the analgesic effect of systemic administration of morphine is mainly mediated through supraspinal opioid system, since the peripheral route of morphine in spinalized rats was practically inactive (33). These behavioral findings suggest that the main central site of the antinociceptive effects of [D-Arg^2]-dermorphin tetrapeptide analogs would be located in the spinal level rather than in the supraspinal level as compared with morphine Therefore, the lack of cross-tolerance to TDAPG-NH_2 or TDAPA may be partially explained by the difference of the main central site of action between the peptides and morphine

Tolerance to and dependence on opioid drugs are also related to opioid receptor subtypes. It is more likely and more robust that mu and delta receptors appear to be involved in opioid tolerance and dependence (7, 20, 26, 27). A previous opioid receptor binding assay in rat brain using [^{3}H]-naloxone as the tracer ligand indicated that [D-Arg^2]-dermorphin tetrapeptide analogs, H-Tyr-D-Arg-Phe-Gly-OH and H-Tyr-D-Arg-Phe-Sar-OH, had 5.3- and 25.5-fold higher affinities than morphine (24) Similarly, TDAPA also showed high affinity in the radioreceptor assay utilizing

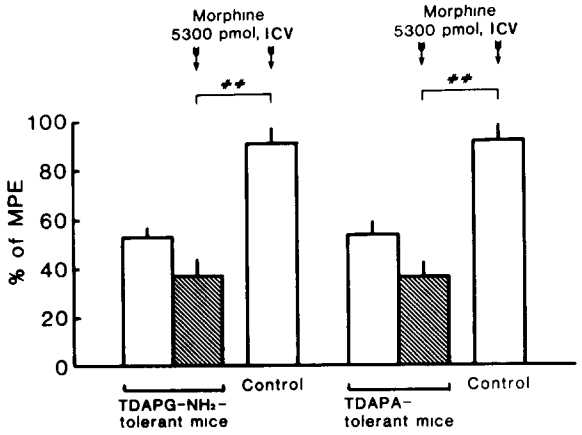

FIG 5 Cross-tolerance to ICV-administered morphine in mice made tolerant to TDAPG-NH_2 and TDAPA Each open column in TDAPG-NH_2- and TDAPA-tolerant mice indicates the value on the 5th day following successive administrations of each peptide Significant differences between ICV morphine in peptide-tolerant mice and ICV morphine in control mice are indicated by ##$p<0$ 01 For other details see Fig 4

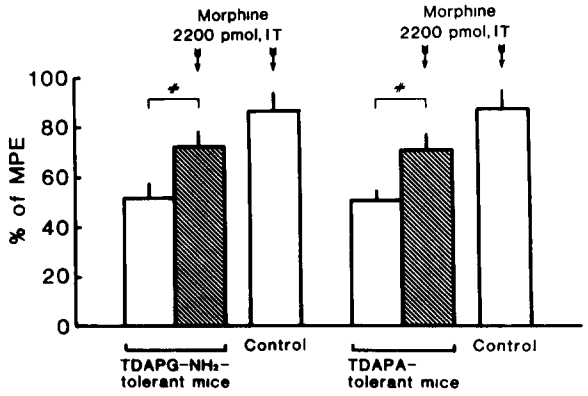

FIG 7 Cross-tolerance to IT-administered morphine in mice made tolerant to TDAPG-NH_2 and TDAPA Each open column in TDAPG-NH_2- and TDAPA-tolerant mice indicates the value on the 5th day following successive administrations of each peptide Significant differences between the 5th day following successive administrations of each peptide and IT morphine in peptide-tolerant mice are indicated by #$p<0$ 05 For other details see Fig 4

radiolabeled dihydromorphine (unpublished data). It is conceivable that [D-Arg^2]-dermorphin tetrapeptide analogs as well as morphine have high affinity for mu opioid receptors in the central nervous system (CNS). These results suggest that both dermorphin analogs and morphine are acting at the mu receptor and the lack of cross-tolerance is a result of the difference in the level of the CNS at which the substances have their primary action. On the other hand, kappa receptor agonists such as U-50488H or spiradoline did not produce cross-tolerance to the antinociception in morphine-tolerant animals (29,30). In our preliminary study, TDAPG-NH_2 and TDAPA produced sedative action similar to kappa agonists, but they have no narcotic antagonist properties such as kappa agonists in both tail-pressure and guinea pig ileum (GPI) assays. It is possible that TDAPG-NH_2 and TDAPA may be partially mediated through kappa receptor.

The present study showed that a marked tolerance to SC- and ICV-administered morphine was observed in TDAPG-NH_2- and TDAPA-tolerant mice, but no significant decrement in the antinociception of IT morphine was seen in mice made tolerant to TDAPG-NH_2 and TDAPA. This observation suggests that the spinal cord is not a major site for the development of tolerance to morphine However, some investigators strongly suggest that the spinal cord plays an important role in development of tolerance to systemic morphine (1, 9, 10, 34) This discrepancy may be partially related to the differences of materials and method, for example, direct lumbar administration in intact mice, IT catheterized rats or spinalized rats. Spinal as well as supraspinal mechanisms are also involved in the induction of tolerance (18,32). It has also been reported that the development of tolerance and dependence is accompanied by changes in the metabolism of dopamine, noradrenaline and serotonin, and in the function of monoaminergic neurons (16, 21, 25, 28, 31, 35) and GABAergic neurons (6).

In summary, the present results clearly showed that no tolerance to antinociception of SC-, ICV- and IT-administered [D-Arg^2]-dermorphin tetrapeptide analogs developed in mice made tolerant to morphine

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