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Comparison of Tizanidine and Morphine With Regard to Tolerance-Developing Ability to Antinociceptive Action

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NABESHIMA, T., S. YAMADA, A. SUGIMOTO, K. MATSUNO AND T. KAMEYAMA. *Comparison of tizanidine and morphine with regard to tolerance-developing ability to antinociceptive action.* PHARMACOL BIOCHEM BEHAV **25**(4) 835–841, 1986.—The antinociceptive, tolerance-developing and anti-withdrawal activities of tizanidine [5-chloro-4-(2-imidazolin-2-yl-amino)-2,1,3-benzo-thiadiazole] were investigated by comparing its effects with those of morphine and clonidine in tail-flick-, hot plate-, acetic acid-induced writhing-, and naloxone-precipitated withdrawal jumping-tests. The antinociceptive action of tizanidine was not altered by naloxone, while that of morphine was antagonized. Tolerance to the tizanidine-induced antinociceptive action and to motor incoordination was developed by successive administration of tizanidine. In the tizanidine-tolerant mice, the antinociceptive action of morphine was significantly decreased, but not sleeping time induced by pentobarbital. The action of tizanidine was not modified in the morphine-tolerant mice. Tizanidine failed to induce morphine-withdrawal jumping and to inhibit naloxone-precipitated withdrawal jumping in the morphine-dependent mice. Cross tolerance to the antinociceptive action induced by tizanidine and clonidine was developed. These results suggest that α_2-adrenoreceptors may be involved in the action mechanism of tizanidine, but not opioid receptors. Functional tolerance to tizanidine action may be developed by successive administration of tizanidine.

Tizanidine Clonidine Morphine Antinociceptive action Tolerance Withdrawal

MORPHINE is an effective pain reliever currently available to the physician and it is the standard against which other analgesics are evaluated. However, the use of narcotics like morphine is hampered by the rapid onset of drug dependence which limits their application. Current research in analgesics is aimed at finding a nonnarcotic analgesic agent which will alleviate pain without the development of physical dependence and which will be able to block withdrawal symptoms in patients currently receiving narcotic drugs. The antihypertensive agent clonidine was reported to block pain-induced volcalization afterdischarge [17] and to reduce certain withdrawal signs in morphine-treated animals [3, 4, 12].

Tolerance to several potent analgesics can be developed [18]. The development of tolerance to clonidine-induced antinociceptive action [16], the sedative effect [10] and suppression of operant behaviour [11], has also been reported.

Kameyama *et al.* [6] have reported that tizanidine [5-chloro-4-(2-imidazolin-2-yl-amino)-2,1,3-benzo-thiadiazole] has an imidazoline structure in common with that of clonidine and inhibits the nociceptive responses induced by chemical, thermal, mechanical and electrical stimuli at dose levels much lower than those required for muscle relaxant action. The action induced by both tizanidine and clonidine was antagonized by yohimbine, an α_2-adrenoreceptor blocker [8]. Moreover, tizanidine decreases body temperature of rats [7]. In addition, tizanidine, when administered intracerebroventricularly, has been shown to possess the capacity for antinociceptive activity [15]. Furthermore, the antinociceptive action of tizanidine is attenuated in spinal mice as is that of morphine [15]. The antinociceptive activity of tizanidine has been found to be stronger than that of morphine in various analgesic tests as is that of clonidine [15]. Therefore, the antinociceptive action of tizanidine may be mediated by the central nervous system.

In present experiments, we have evaluated tizanidine for its antinociceptive, tolerance-developing and anti-withdrawal activities by comparing its effects with those of morphine and clonidine in the same test.

METHOD

Animals and Environment

Male albino ddY mice (Shizuoka Laboratory Animal Center, Shizuoka, Japan) weighing 18–22 g were used. The mice were allowed food and water ad lib. Environmental conditions were constant at 23±1°C, 50±5% humidity with a 12 hr light-dark cycle.

Tail-Flick-Test

The method used was in accordance with that described by D'Amour and Smith [2]. The time until the mouse flicked

TABLE 1

DEVELOPMENT OF TOLERANCE TO TIZANIDINE-INDUCED ANTINOCICEPTIVE ACTION IN TAIL-FLICK-TEST BY SUCCESSIVE ADMINISTRATIONS OF TIZANIDINE

Days of administration	0	5	7	10	12
Tail-flick latency (sec)[a]	11.4 ± 1.30	8.59 ± 0.82	8.05 ± 1.49	6.66 ± 1.42*	5.69 ± 1.06*

Mice whose tail-flick latency were between 1.5 and 4 sec were selected and were successively administered tizanidine (0.5 mg/kg, SC) twice a day for 0, 5, 7, 10 and 12 days.

[a]Individual groups of mice were used for each time point and challenged with tizanidine (1 mg/kg, SC) 24 hr after the last of 5, 7, 10 and 12 successive injections of tizanidine and the tail-flick latency was recorded 30 min after the challenge of tizanidine.

N=9–10 in each group.

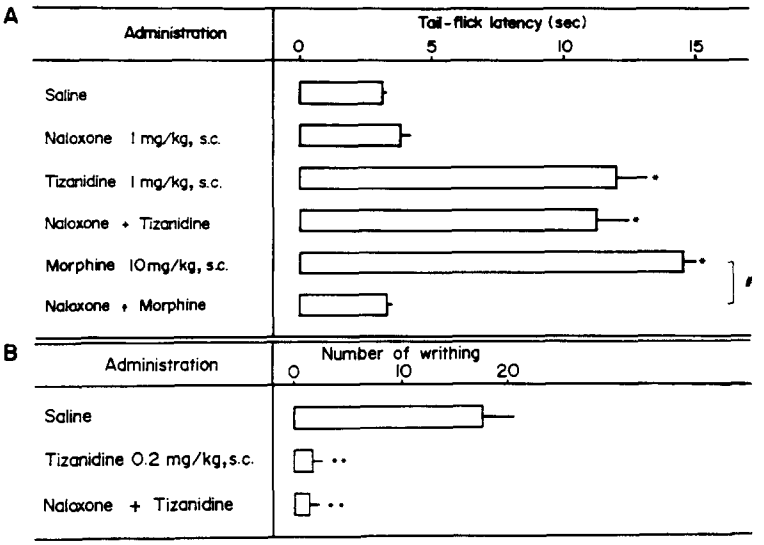

FIG. 1. Effect of naloxone on the antinociceptive action of tizanidine and morphine in mice. Mice were given naloxone (1 mg/kg, SC) 15 min before treatment with tizanidine or morphine. A: tail-flick-test, B: acetic acid-induced writhing-test. N=10–20 in each group. *$p<0.01$ vs. saline, #$p<0.01$ vs. morphine alone.

its tail out of the path of a beam of radiant heat was recorded twice before administration of the test drugs. Mice whose reaction times were between 1.5 and 4 sec were selected. A cutoff time of 15 sec was used. Pain threshold was expressed as response time (sec) taken for animals to flick their tails. The percentage of inhibition was calculated as follows: % of inhibition = 100 × (A − B)/15 − B, A = reaction time of the drug-treated animals, B = reaction time of the control animals.

Acetic Acid-Induced Writhing-Test

Acetic acid-induced writhing-test was performed on mice following the method of Koster *et al.* [9] with minor modification. Acetic acid (0.7%) solution was injected intraperitoneally (IP) 20 min after administration of the test drugs. Complete extension of either hind limb was regarded as a writhing response. The incidence of writhing was recorded during a period of 10 min, commencing 30 min after administration of the test drugs. The degree of inhibition was calculated as follows: % of inhibition = 100 × (A − B)/A; A: incidence of writhing in the control group; B: incidence of writhing in the drug-treated group.

Hot Plate-Test

The method used was based on that described by Woolfe and Macdonald [21]. Mice were placed on a hot plate at the temperature of 55±0.5°C. Animals licking or shaking a hind foot within 3–7 sec after being placed on the hot plate were selected. Animals showing a doubling of their pre-drug latency were regarded as showing a positive antinociceptive effect.

Motor Coordination-Test

Motor incoordination was defined as a loss of the ability to stay on 6 mm square rods for 30 sec and expressed as the percentage of the animals which fell off with respect to the total number of animals tested in each group [5].

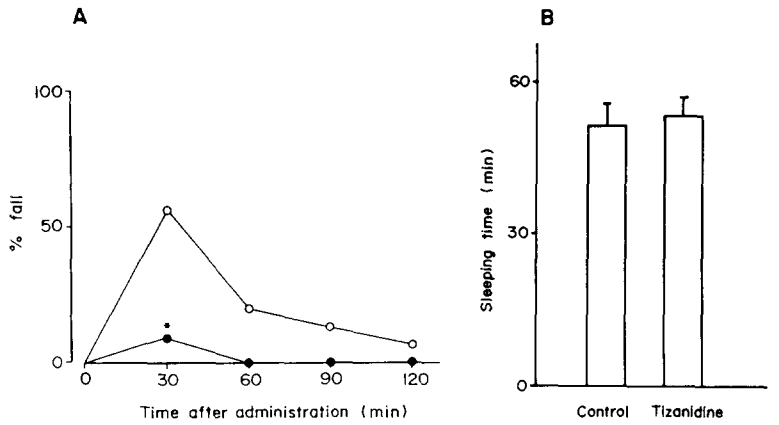

FIG. 2. Development of tolerance to tizanidine-induced motor incoordination (A) by successive administration of tizanidine and its effect on pentobarbital-induced sleeping (B) in mice. Tolerance to tizanidine-induced motor incoordination (N=15–22 in each group) was developed and the pentobarbital sleeping time (N=10 in each group) was recorded as described in the Method section. ○: tizanidine (35 mg/kg) single administration; ●: tizanidine successive administration. *$p<0.01$ vs. tizanidine once.

Sleeping Time by Acute Administration of Sodium Pentobarbital

Mice were challenged with sodium pentobarbital (60 mg/kg, IP) 24 hr after 5 days' successive administration of tizanidine (35 mg/kg, SC, twice a day), and sleeping times of individual animals were recorded. The sleeping time was the elapsed time between the time the animal lost righting reflex and the time the animal righted itself spontaneously [13]. Each group contained 10 mice.

Development of Tolerance

To investigate the development of tolerance to tizanidine-induced antinociceptive action (ED_{50} = 0.5 mg/kg, SC) in the tail-flick-test (Table 1) and to motor incoordination induced by tizanidine (ED_{50} = 35 mg/kg, SC) (Fig. 2), twice the ED_{50} dose of drug for each response was administered successively and the development of tolerance was evaluated 24 hr after the last injection. Figures 3 and 5 show the results of our experiment to ascertain potential cross-tolerance to morphine or clonidine; each group of mice was given high dose of SC tizanidine (35 mg/kg), morphine (350 mg/kg) or clonidine (20 mg/kg) twice a day for 5 days to rapidly develop tolerance to each drug. Mice in each group were divided into three sub-groups 24 hr after the last injection. The mice in each sub-group were challenged with tizanidine, morphine or clonidine at twice the ED_{50} dose of drug for antinociceptive action of each drug and the antinociceptive action of test drugs was evaluated by tail-flick-, hot plate- or acetic acid-induced writhing-tests.

In the withdrawal experiment, morphine-tolerant mice were given naloxone (5 mg/kg, SC) and/or tizanidine and the occurrence of withdrawal jumping was recorded for 15 min after the injection of naloxone as an all-or-none response.

Drugs

The following drugs were used: tizanidine hydrochloride (Sandoz), morphine hydrochloride (Shionogi), clonidine hydrochloride (TokyoKasei), naloxone hydrochloride (Endo Labo.) and sodium pentobarbital (Abbott Labo.). Since the peak of the antinociceptive activity of tizanidine was observed at 30 min after subcutaneous administration, analgesic tests took place 30 min after the administration.

Statistical Analysis

The statisitcal significance of the data was evaluated using Mann-Whitney's U-test.

RESULTS

Effect of Naloxone on the Antinociceptive Action of Tizanidine and Morphine

The antinociceptive action of tizanidine and morphine in the tail-flick or acetic acid-induced writhing-tests was shown in Fig. 1. Tizanidine (1 mg/kg, SC) and morphine (10 mg/kg, SC) significantly prolonged the tail-flick latency. Naloxone, an opiate antagonist (1 mg/kg, SC) failed to antagonize the action of tizanidine or alter the intrinsic pain threshold, while it completely inhibited the action of morphine (Fig. 1A) in agreement with our previous report [6]. Tizanidine (0.2 mg/kg, SC) strongly inhibited the acetic acid-induced writhing (Fig. 1B). The action of tizanidine was not antagonized by naloxone (1 mg/kg, SC).

Development of Tolerance to Tizanidine-Induced Antinociceptive Action by Successive Administration of Tizanidine at Low Dose Levels

As shown in Table 1, acute tizanidine (1 mg/kg, SC) significantly prolonged the tail-flick latency in the control group. When mice were successively given tizanidine (0.5 mg/kg, SC), the antinociceptive action induced by tizanidine (1 mg/kg) was gradually decreased. After the 10 days-successive administration of tizanidine the degree of increase in tail-flick latency of mice which received the tizanidine challenge was significantly decreased compared to the control group.

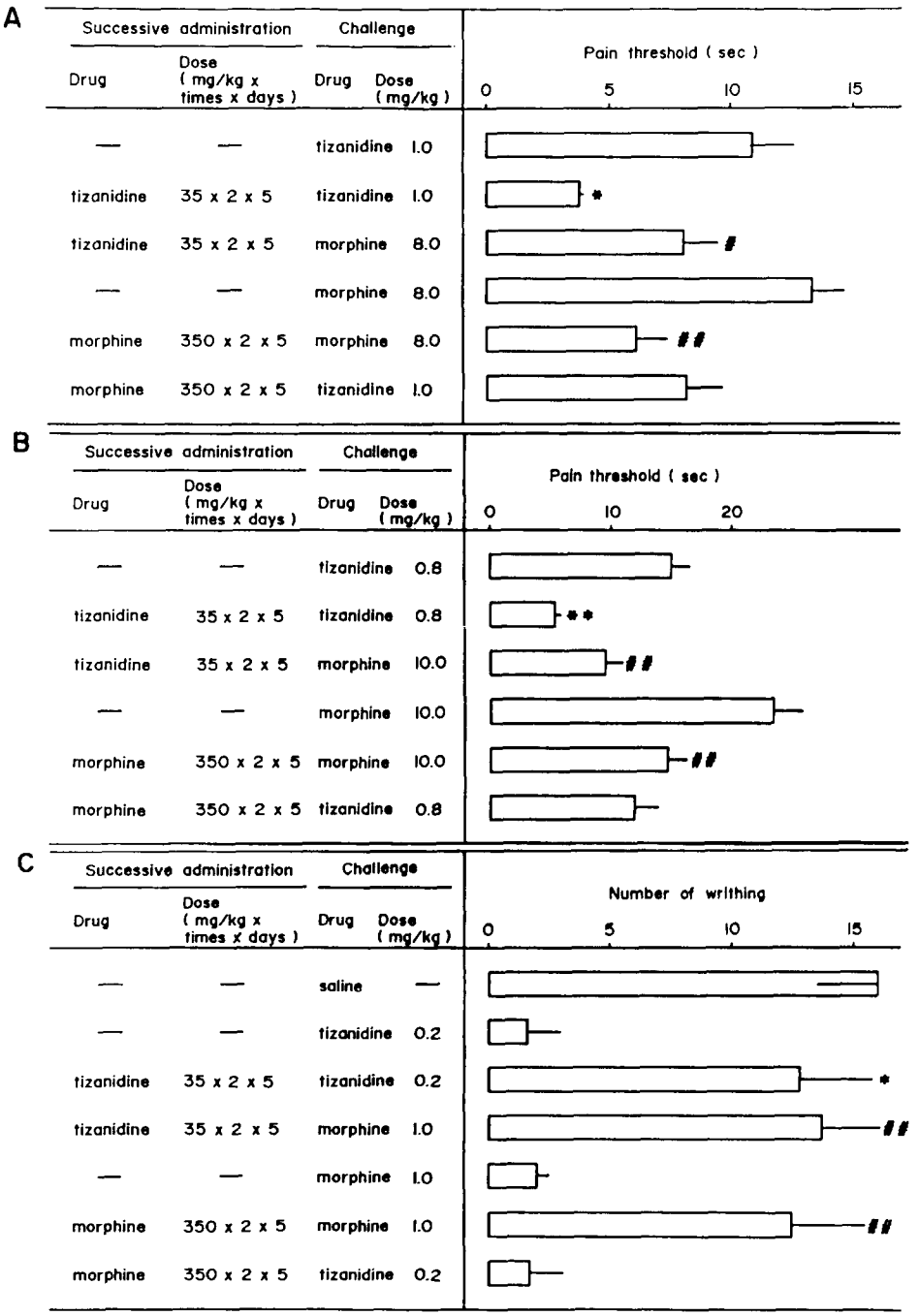

FIG. 3. Development of tolerance to tizanidine- and morphine-induced antinociceptive action by successive administrations of tizanidine in the tail-flick (A)-, hot plate (B)- and acetic acid-induced writhing (C)-tests. Tolerance to antinociceptive action induced by tizanidine and morphine was developed according to the schedule described in the Method section. The antinociceptive action of tizanidine and morphine was evaluated 30 min after the challenge of each drug at a dose of $2 \times ED_{30}$ in each test. N=6–8 in each group. $^{*}p<0.01$ vs. tizanidine single administration. $\#p<0.05$, $\#\#p<0.01$ vs. morphine single administration.

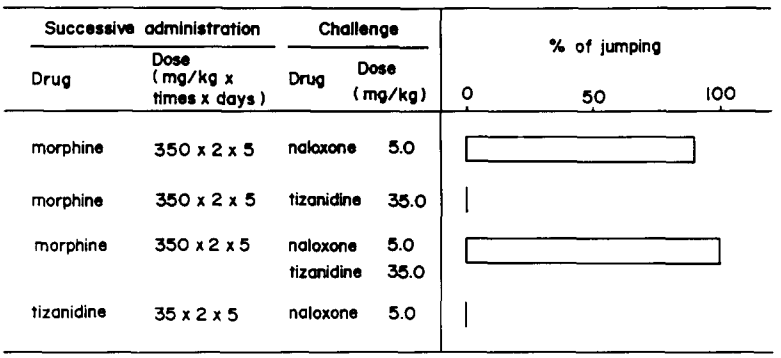

FIG. 4. Effect of tizanidine on naloxone-precipitated jumping in the morphine dependent mice. Naloxone-precipitated jumping was recorded as described in the Method section. Tizanidine was simultaneously administered in combination with naloxone. N=10 in each group.

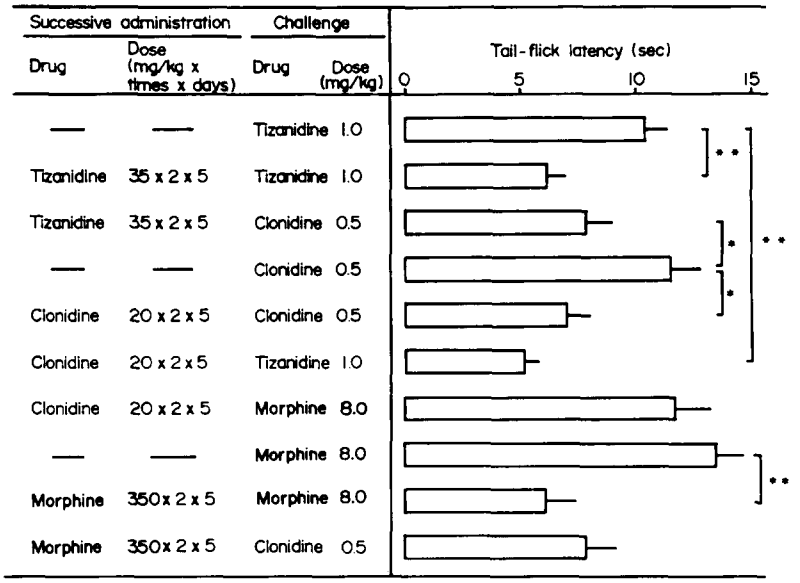

FIG. 5. Cross tolerance to the antinociceptive action induced by tizanidine and clonidine. Tolerance to tizanidine-, clonidine- and morphine-induced antinociceptive action was developed as described in the Method section. The antinociceptive action of tizanidine, clonidine and morphine was evaluated 30 min after the administration of each drug at a dose of $2\times ED_{50}$ in the tail-flick-test. N=8–10 in each group. $*p<0.05$, $**p<0.01$ vs. single administration.

Development of Tolerance to Tizanidine-Induced Motor Incoordination by Successive Administration of Tizanidine at High Dose Levels

Tizanidine-induced motor incoordination was investigated in the mice given tizanidine (35 mg/kg, SC: ED_{50} for motor incoordination) twice a day for 5 days, in order to determine whether chronic tizanidine could produce tolerance to the other pharmacological action induced by tizanidine. As shown in Fig. 2A, acute tizanidine (35 mg/kg) produced motor incoordination in over 50% of the mice in the control group at the peak of the action and the tizanidine action was observed for 2 hr. In the mice treated with tizanidine for 5 days, the action of tizanidine was significantly decreased in terms of potency and duration.

Pentobarbital-Induced Sleeping Time for Mice Treated Successively With Tizanidine

To investigate whether the hepatic microsomal drug-metabolizing enzyme system was involved in the development of tolerance to tizanidine-induced action, sleeping time induced by pentobarbital in the control group was compared to that in tizanidine-tolerant group, since pentobarbital-induced sleeping time will be decreased when hepatic drug metabolizing enzyme system is induced by the successive

tizanidine treatment. As shown in Fig. 2B, pentobarbital (60 mg/kg, IP)-induced sleeping time in the tizanidine-tolerant group was not different from that in the control group.

Development of Tolerance to the Antinociceptive Action of Tizanidine and Morphine in the Tail-Flick-, Hot Plate- and Acetic Acid-Induced Writhing-Tests and Cross-Tolerance to Morphine

To produce a high degree of tolerance rapidly, the injection schedule for the motor incoordination experiment was employed. Since ED_{50} in the motor coordination-test was about 70 times higher than that in the tail-flick test, the corresponding dose of morphine was administered successively for 5 days. At the dose of $2\times ED_{50}$, acute tizanidine and morphine produced significant antinociceptive action in the tail-flick (Fig. 3A)-, hot plate (Fig. 3B)- and acetic acid-induced writhing (Fig. 3C)-tests. The successive administration of tizanidine and morphine developed tolerance to the antinociceptive action of tizanidine and morphine, respectively, in the tests used. In the tizanidine-tolerant mice, the antinociceptive action of morphine in the tail-flick-, hot plate- and acetic acid-induced writhing-tests was significantly attenuated. On the contrary, the action of tizanidine did not change in the morphinetolerant mice in any tests.

Effect of Tizanidine on Morphine-Withdrawal Jumping

To confirm the differences of action mechanism between tizanidine and morphine, the effect of tizanidine on morphine-withdrawal jumping in mice was investigated. In addition, we attempted to investigate whether naloxone produces "tizanidine-withdrawal jumping" in the tizanidine-tolerant mice. As shown in Fig. 4, naloxone (5 mg/kg, SC)-induced jumping in the morphine-tolerant mice. Tizanidine neither inhibited the jumping produced by naloxone nor induced jumping in the morphine-tolerant mice. Naloxone failed to induce "tizanidine-withdrawal jumping".

Cross-Tolerance to the Antinociceptive Action of Tizanidine and Clonidine

To clarify the similarity of the mechanisms of antinociceptive action of tizanidine and clonidine, but not morphine, the antinociceptive action of tizanidine and morphine in the clonidine-tolerant mice was investigated. As shown in Fig. 5, tolerance to the antinociceptive action of clonidine was developed by the 5 days' successive administration of clonidine. In the clonidine-tolerant mice, the antinociceptive action of tizanidine was significantly decreased, but not that of morphine. The action of clonidine was also significantly attenuated in the tizanidine-tolerant mice. However, the antinociceptive action of clonidine did not change in the morphine-tolerant mice.

DISCUSSION

We have shown in earlier studies that tizanidine structurally resembles clonidine and produces antinociceptive action which is greater than that of morphine in various analgesic tests [6], but which is smaller than that of clonidine in the tail-flick- and acetic acid-induced writhing-tests [8]; this agrees with present results. It is apparent that the antinociceptive activity of tizanidine is not mediated through a narcotic or endorphin mechanism, though tizanidine has a potent antinociceptive action compared to morphine. In all instances, naloxone failed to antagonize the action of tizanidine. Kameyama *et al.* [8] have reported that α_2-adrenoreceptors may be of importance in mediating tizanidine-induced antinociceptive action in the tail-flick-test, since the tizanidine action is antagonized completely by an α_2-adrenoreceptor blocker (yohimbine), but not by an α_1-adrenoreceptor, dopamine receptor, serotonin receptor or opiate receptor blockers. The mechanism of action of tizanidine described above is similar to that of clonidine [8,17]. However, there is an interaction of opioid and α-adrenoreceptor systems, since clonidine is able to inhibit some precipitated withdrawal syndromes in morphine-dependent rats [4, 19, 20], but this effect of clonidine is not antagonized by naloxone [19]. In addition, acute administration of clonidine enhances the antinociceptive action of morphine and the morphine action is reduced in the clonidine-tolerant rats [16]. Therefore, the relationship between tizanidine and the opioid system was investigated in the present experiments in more detail.

In agreement with previous results [6,8], naloxone failed to antagonize the antinociceptive action of tizanidine in the tail-flick- and acetic acid-induced writhing-tests, while it completely antagonized that of morphine. Furthermore, the action of tizanidine was not altered in the morphine-tolerant mice in the tail-flick-, hot plate- and acetic acid-induced writhing-tests. Moreover, tizanidine was not able to suppress the naloxone-precipitated withdrawal jumping in the morphine-dependent mice or produce the morphine-withdrawal jumping. The difference in the ability to inhibit precipitated withdrawal syndrome in morphine-dependent animals between tizanidine and clonidine may be due to the differences in the affinity of both drugs to α_2-adrenoreceptors and the differences among in the species used. In addition, naloxone failed to produce any withdrawal syndromes in the tizanidine-tolerant mice. These results suggest that tizanidine does not act directly to either stimulate or block opioid receptors.

The antinociceptive action of morphine in the tail-flick-, hot plate- and acetic acid-induced writhing-tests was decreased in the tizanidine-tolerant mice. There are indications that the α-agonists and antagonists can act in some way on opioid receptors: piperoxan has between one-third and one-quarter of the potency of naloxone in antagonizing morphine [1]. In addition, pretreatment of mice with a single dose of clonidine causes a marked reduction in the potency of a subsequent dose of morphine, while the antinociceptive action of morphine is potentiated when clonidine is given simultaneously with morphine [1]. The findings of the present study further emphasize the link between opioid and α-adrenoreceptors. One possible explanation for these findings is that an opioid receptor is linked to its effector mechanism through an α-adrenoreceptor, and that opioid agonists in some way activate the α-adrenoreceptor. This theory would accomodate findings that the morphine action can be markedly attenuated in the tizanidine-tolerant mice, though the tizanidine action is not able to change in the morphine-tolerant mice. There were no differences between the sleeping time induced by pentobarbital in tizanidine-tolerant mice and that in non-tolerant mice. It is well known that pentobarbital can be metabolized by the hepatic drug metabolizing enzyme system and induction of this system is one of the mechanisms of tolerance development [14]. Therefore, shortening of sleeping time induced by pentobarbital is an indicator of the induction of the drug metabolizing system.

Taken together, the present results suggest that the development of tolerance to tizanidine action is dependent on the central nervous systems, but not the hepatic drug metabolizing system. There was a discrepancy between the results obtained by present authors and the results obtained by previous authors [16] concerning the antinociceptive action of morphine in the clonidine-tolerant animals. This discrepancy may be due to differences in the species used, the schedule for developing the tolerance and the method for evaluating tolerance. In addition, this discrepancy suggests that the action mechanism of tizanidine is for the most part similar to that of clonidine, but slightly different since the antinociceptive action of morphine was reduced in the tizanidine-tolerant mice, but not in the clonidine-tolerant mice.

Cross tolerance to the antinociceptive action was developed between tizanidine and clonidine: the action of tizanidine was attenuated in the clonidine-tolerant mice and vice versa. The result supports our hypothesis that an α_2-adrenoreceptor may be involved in the action mechanism of tizanidine [8].

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