Single Dose Tolerance to the Analgesic Effect of Clonidine and Cross-Tolerance between Morphine and Clonidine

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Abstract—A single dose of clonidine developed tolerance to its analgesic effect. The tolerance reached its peak acutely on the 2nd day and lasted more than 5 days. Neither the analgesic effect nor the development of tolerance was modified by the pretreatment with naloxone. On the 2nd day, clonidine tolerant animals were also tolerant to morphine, but morphine tolerant animals, after a single dose of morphine on the 1st day, were not tolerant to clonidine. On the 5th day, however, clonidine tolerant animals were tolerant to morphine, and vice versa. Thus, the interaction between morphine and clonidine was “one-way” on the 2nd day, and cross-tolerance was only demonstrated on the 5th day. With a treatment with clonidine plus naloxone on the 1st day, the development of cross-tolerance to morphine was completely suppressed on the 2nd day but not on the 5th day. These results confirmed our previous finding that acute and delayed tolerance are different in nature, and the development of tolerance to morphine and clonidine are partially overlaid with a common mechanism which is not mediated by opioid receptors.

The potent analgesic effect of clonidine, an α -adrenergic agonist, has been widely recognized in experimental animals (1–7) and in man (8). The antinociceptive effect of clonidine is not antagonized by naloxone (2, 5, 6), suggesting that the effect is not mediated by opioid receptors. Non-participation of opioid receptors is also demonstrated by binding assay using ^3H -clonidine (9). On the other hand, development of tolerance to the effect is still a subject of controversy. Chance (10), Paalzow (11) and Takahashi et al. (7) have reported the development of tolerance to the analgesic effect, but Ramaswamy et al. (12) and Lipman and Spencer (13) failed to demonstrate the tolerance. The animals tolerant to clonidine were also tolerant to morphine (11); however, morphine tolerant animals were not tolerant to clonidine (6). The “one-way” interaction between morphine and clonidine has also been demonstrated after a single dose of the drugs (14).

We have reported that a single dose of morphine developed two different types of

tolerance to its analgesic effect, acute and delayed types, and the formation of the former is prevented by naloxone, but the latter is insensitive to the treatment (15). Based on these results, to clarify the discrepancies between previous papers concerning tolerance and cross-tolerance, a precise study was undertaken on the development of cross-tolerance between morphine and clonidine after a single dose of the drugs.

Materials and Methods

Animals: Male mice of the dd strain weighing 18 to 20 g were purchased and housed as a group of 20 to 25 animals in a plastic cage. They were kept in a room at an ambient temperature of $22 \pm 1^\circ\text{C}$ and given normal laboratory diet and tap water ad libitum. After reaching 23 to 25 g, they were randomly divided into groups of 10 animals and used for experiments.

Drugs: The following drugs were used: morphine-HCl, naloxone-HCl (gift from National Institute of Drug Abuse, U.S.A.) and clonidine-HCl (gift from Boehringer-

Ingelheim). The drugs were dissolved in saline and administered i.p. in a volume of 0.1 ml/10 g of body weight. The dose was expressed in terms of the salt.

Assessment of analgesic effect: The analgesic effect (the response time, a cut-off time of 6 sec) was measured by a modified Haffner's method (16).

Evaluation of tolerance: On the 1st day, animals were treated with various doses of morphine or clonidine. The degree of tolerance was assessed by measuring the analgesic effect of the test dose of morphine (10 mg/kg) or clonidine (1 mg/kg) on the 2nd, 5th and 10th day. The measurement was made every 15 min for 1.5 hr. The effect was calculated by plotting the increase of response time (sec) against time (min), and the area under the curve (AUC) was expressed as percent of the effect obtained in saline treated controls.

Evaluation of cross-tolerance: Animals were treated with morphine (50 mg/kg) or clonidine (5 mg/kg) on the 1st day. To evaluate the development of cross-tolerance, the test dose of clonidine was administered to morphine treated animals, and the test dose of morphine was administered to the clonidine treated animals.

Effect of naloxone on the development of tolerance and cross-tolerance: Animals were pretreated with naloxone (10 mg/kg, 30 min prior to the injection of morphine or clonidine) on the 1st day, and tests were performed on the 2nd and 5th day. All the tests on tolerance and cross-tolerance were made in a separate group of animals.

Statistical analysis: Statistical significances were evaluated by Student's *t*-test.

Results

Analgesic effect and development of tolerance: Morphine produced a potent and long-lasting analgesic effect in a dose-dependent manner, from 10 to 50 mg/kg. The analgesic effect of clonidine was also dose-dependent up to 5 mg/kg, and about 10 times more potent than morphine. Five mg/kg of clonidine elicited nearly equipotent analgesia with 50 mg/kg of morphine; however, a high dose, 10 mg/kg, showed less effect (Fig. 1).

Fig. 1. Analgesic effect of morphine and clonidine. The effect (response time, a cut-off time of 6 sec) was measured by a modified Haffner's method. Morphine: 50 (○—○), 30 (●—●) and 10 (○—○) mg/kg. Clonidine: 10 (×—×), 5 (■—■), 2.5 (■—■) and 1 (□—□) mg/kg. Drugs were administered i.p., and the data are the mean of 20 to 40 animals.

Tolerance developed acutely on the 2nd day after morphine injection, and the degree of the tolerance was dependent on the dose of the initial treatment. The tolerance developed after a high dose of morphine, 30 and 50 mg/kg, was further intensified, reaching a peak on the 5th day, and gradually disappeared. Tolerance to clonidine also developed dose-dependently on the 2nd day. The peak was on the 2nd day in the animals treated with a high dose, 2.5 and 5 mg/kg, and it was on the 5th day in the group treated with 1 mg/kg. The tolerance disappeared somewhat more rapidly than that of morphine (Fig. 2).

Thus, in the following experiments, the dose of morphine and clonidine were fixed as 50 and 5 mg/kg, respectively, and the tests were made on the 2nd and 5th day.

Effect of naloxone on the development of tolerance to morphine: Naloxone, 10 mg/kg, given 30 min prior to the injection of morphine partially suppressed the analgesic effect, especially the early period of the effect. Partial masking of morphine analgesia by naloxone on the 1st day inhibited the development of tolerance on the 2nd day, acute tolerance, but could not prevent the development of tolerance on the 5th day, delayed tolerance (Fig. 3).

Effect of naloxone on the development of

Fig. 2. Development and disappearance of tolerance after a single dose of morphine and clonidine. On the 1st day, animals were treated with various doses of morphine or clonidine, and the analgesic effect of the test dose of morphine, 10 mg/kg, or clonidine, 1 mg/kg, was estimated on the 2nd, 5th and 10th day. Symbols are indicated in the footnote of Fig. 1.

Fig. 3. Effect of naloxone on the development of tolerance to morphine. On the 1st day, animals were given morphine, 50 mg/kg, with (●—●) or without (○—○) pretreatment of naloxone, 10 mg/kg, 30 min before morphine administration. The analgesic effect of the test dose of morphine, 10 mg/kg, was estimated on the 2nd and 5th day. For evaluation of tolerance, AUC was calculated and expressed as percent of the effect of control groups. Tests were performed in separate groups which consisted of at least 20 animals. Treated with morphine (▨) or morphine plus naloxone (□) on the 1st day.

tolerance to clonidine: Naloxone did not antagonize the analgesic effect of clonidine and had no influence on the development of tolerance, both acute and delayed types (Fig. 4).

Fig. 4. Effect of naloxone on the development of tolerance to clonidine. On the 1st day, animals were given 5 mg/kg clonidine, with (■—■) or without (□—□) pretreatment of naloxone. The analgesic effect of the test dose of clonidine, 1 mg/kg, was estimated on the 2nd and 5th day. Treated with clonidine (□—□) or clonidine plus naloxone (■—■) on the 1st day. For other details, see the footnote of Fig. 3.

Fig. 5. Effect of naloxone on the development of cross-tolerance between morphine and clonidine. On the 1st day, animals were treated with (a) 50 mg/kg morphine or (b) 5 mg/kg clonidine with (▨) or without (□) 10 mg/kg naloxone 30 min before the injection of the respective drugs. The analgesic effect of the test dose of morphine or clonidine was measured on the 2nd and 5th day. The analgesic effect of the test dose of morphine in morphine tolerant animals (▨) or that of clonidine in clonidine tolerant animals (▨) after a single dose of the respective drugs on the 1st day.

Development of cross-tolerance between morphine and clonidine: In the animals treated with morphine or morphine plus naloxone on the 1st day, clonidine elicited a similar degree of analgesia on the 2nd day as

in control animals, but the effect was significantly weakened on the 5th day to the same extent as in clonidine tolerant animals. Thus, the cross-tolerance to clonidine was only developed on the 5th day. On the other hand, in the animals treated with clonidine on the 1st day, the analgesic effect of morphine was attenuated both on the 2nd and 5th day, indicating the development of cross-tolerance to morphine. However, the treatment with clonidine plus naloxone on the 1st day, completely suppressed the development of cross-tolerance to morphine on the 2nd day, but failed to modify the development of cross-tolerance to morphine on the 5th day (Fig. 5).

Discussion

We have reported that a single large dose of morphine produced two different types of tolerance, acute and delayed types, and the formation of the former is inhibited when the analgesic effect of morphine on the 1st day was masked completely or partially with 3 hourly injections of naloxone starting from just before or at 1 or 2 hr after morphine administration (15). Similarly, partial inhibition of the early period of morphine analgesia by a single dose of naloxone, given 30 min before morphine injection, also inhibited the development of acute tolerance. These results suggest that for the development of acute tolerance after a single dose of morphine, full expression of the morphine effect at the initial treatment is essential. On the other hand, as far as the delayed tolerance is concerned, naloxone treatment could not modify the development, indicating the participation of mechanisms unrelated to opioid receptors.

A single dose of clonidine also developed tolerance nearly equipotent and somewhat short duration compared with morphine. However, naloxone could modify neither the analgesic effect nor the development of tolerance. These results indicate that the effect of clonidine is not mediated by opioid receptors.

Clonidine tolerant animals are also tolerant to morphine on the 2nd and 5th day of clonidine treatment. Contrariwise, the animals tolerant to morphine were not tolerant to clonidine on the 2nd day. The "one-way"

interaction between morphine and clonidine after a single injection has been reported. Bentley et al. described that 3 hr after pretreatment of mice with a single dose of clonidine caused a marked reduction in the potency of a subsequent dose of morphine, but pretreatment with morphine had only a slight effect on clonidine analgesia (14). In the present experiments, this type of interaction was only observed on the 2nd day of clonidine treatment and not on the 5th day. These facts may support our previous finding that the tolerance which developed on the 2nd and 5th day after a single dose of morphine, namely acute and delayed tolerance, are different from each other in the underlying mechanisms (15). The effect of clonidine was insensitive to naloxone treatment, but when the animals were treated with clonidine plus naloxone on the 1st day, the development of cross-tolerance to morphine was completely suppressed on the 2nd day. Same treatment with clonidine plus naloxone on the 1st day, however, could not affect the development of cross-tolerance to morphine on the 5th day. Naloxone itself did not modify the effect of clonidine on the 2nd and 5th day (data not shown). These results clearly indicate that acute and delayed tolerance are different in nature and on the other hand, the development of tolerance to morphine and clonidine are partially underlaid with a common mechanism which is not mediated by opioid receptors.

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