Morphine Analgesia without Development of Tolerance in Reserpinized Mice

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Abstract—The relationship between the brain monoaminergic mechanism and morphine tolerance was examined in reserpinized mice. In parallel with the reduction of brain monoamine content, the analgesic effect of morphine was reduced in reserpinized animals. At the peak of the reserpine effect, 24 hr after a single dose of 2.5 mg/kg reserpine, i.p., the analgesic effect of morphine was lowered to about 45% of that in naive animals; and 5 days after reserpine treatment, it recovered to about 60% of the control activity. In these animals, the lowered effect of morphine was maintained at the same range during 6 daily repetitions, and the development of tolerance was suppressed. When daily morphine injection was started from 10 days after reserpine treatment, at the time when the brain level of monoamines was still reduced to 60 to 80% of the control, tolerance developed as rapidly as in control animals. On the other hand, daily treatment with a small dose of reserpine, 0.1 mg/kg, neither affected the brain level of norepinephrine and dopamine nor modified morphine analgesia, but completely blocked the development of tolerance. These results may suggest that suppression of the development of tolerance to morphine analgesia is not attributed to the reduction of brain norepinephrine and dopamine by reserpine. Morphine analgesia without development of tolerance in reserpinized mice may indicate the dissociation of the analgesic effect from tolerance liability.

The antagonistic effect of reserpine on the analgesic effect of morphine has been widely accepted, and it is suggested that brain catecholamines, in particular norepinephrine, play a role in the mechanism (1–5). Takagi et al. (1, 5, 6) demonstrated a similar antagonistic effect of tetrabenzine on morphine analgesia. They also reported the suppressive effect of tetrabenzine on the development of tolerance to morphine and suggested the involvement of catecholamines in the mechanism (7). We report here the relationship between the brain monoaminergic mechanism and morphine tolerance in reserpinized mice, and we present evidence which supports our previous finding that morphine analgesia and tolerance to the effect are separable from each other by underlying mechanisms (8).

Materials and Methods

Animals: Male ddY mice weighing 18 to 20 g were purchased and housed in a group of 20 animals in a cage. They were kept in a room maintained at 22±1°C and were given normal laboratory diet and tap water ad libitum. After reaching 23 to 25 g, they were used for experiments.

Determination of brain monoamine level: Animals were treated with a single dose of reserpine, 0.1, 1.25 or 2.5 mg/kg, i.p., and another group of animals were treated daily with 0.1 mg/kg reserpine, i.p., for 10 days. They were sacrificed by decapitation and brain level of monoamines was determined using the reverse phase partition mode of HPLC according to Krstulovic and Powell (9). The Model LC-5A HPLC system
(Shimadzu) was used with a flow rate of 1 ml/min, a reverse phase column (Unisil NQ C18, 4.6x250 mm, GC Kogyo) and a fluorescence detector (RF-530, Shimadzu); the mobile phase was 0.02 M KH$_2$PO$_4$ (pH 3.7) containing 6% MeOH.

Evaluation of analgesic effect: Analgesic effect of 10 mg/kg of morphine, i.p. (the response time, a cut-off time of 6 sec) was measured by the modified Haffner's method (10). The measurement was made every 15 min for 90 min, and the effect was calculated as area under the curve (AUC) by plotting the increase of response time (sec) on the ordinate and time intervals (min) on the abscissa.

Assessment of tolerance: The analgesic effect of 10 mg/kg of morphine was determined daily and expressed as percent of the effect obtained in naive control animals.

Drug administration: Morphine-HCl (Takeda Pharm. Co.) was dissolved in saline, and reserpine (Apoplon, Daiichi Pharm. Co.) was diluted with saline and administered i.p. in a volume of 0.1 ml/10 g of body weight. In the group daily treated with reserpine plus morphine, the initial dose of reserpine was given 24 hr prior to the start of morphine administration; and from the second dose, it was administered after every measurement of morphine analgesia.

Statistical analysis: Values are expressed as the mean±S.E. The statistical significance of difference was assessed by Student's t-test.

Results
Changes in brain level of monoamines
A single dose of reserpine dose-dependently reduced the brain content of monoamines: and 24 hr after injection of 2.5 mg/kg, the level of norepinephrine, dopamine and serotonin were lowered to about 30, 50 and 40% of their control level, respectively. Maximal effect of reserpine was attained by 24 hr and gradually disappeared; however, even after 10 days, the content of the monoamines remained at about 60 to 80% of the control level. A half dose of reserpine, 1.25 mg/kg, produced a moderate effect, and no appreciable changes was observed by 0.1 mg/kg (Fig. 1).

Morphine analgesia and development of tolerance in reserpinized mice
a) Effect of a single large dose of reserpine: In parallel with the reduction of brain

![Graph: Changes in brain level of norepinephrine, dopamine and serotonin after administration of reserpine.](image)

Fig. 1. Changes in brain level of norepinephrine, dopamine and serotonin after administration of reserpine. Brain level of monoamines was determined using the reverse phase partition mode of HPLC after a single dose of 0.1 (○—○), 1.25 (●—●) or 2.5 (□—□) mg/kg of reserpine at the day indicated in the Figure. Data are shown as a percent of the content in naive animals (norepinephrine, 0.34±0.02; dopamine, 1.04±0.13; serotonin, 0.52±0.06 μg/g tissue). Values are the mean of at least 5 animals. **: Significantly different from the control level (P<0.01).
monoamine content, the analgesic effect of morphine was reduced in reserpinized animals. At the peak of the effect, 24 hr after a single injection of 2.5 mg/kg of reserpine, the analgesic effect of morphine was lowered to about 45% of that in naive animals and gradually recovered to around 80% of the control level by 10 days.

When daily injection of morphine was started from day 1, 24 hr after reserpine, development of tolerance was completely blocked, and the analgesic effect of morphine tended to increase during 5 daily repetitions. Five days after reserpine injection, the analgesic effect of morphine was still significantly reduced to about 60% of the control. In these animals, the reduced effect of morphine was maintained almost at the same level during 5 daily repetitions and the development of tolerance was more delayed than that in the control animals. However, when morphine injection was started from 10 days after reserpine treatment, tolerance developed as rapidly as in normal control animals.

In the animals treated with saline instead of reserpine, daily injection of morphine rapidly produced tolerance to its analgesic effect, and after 5 repetitions, the effect of 10 mg/kg of morphine disappeared completely. The analgesic effect of 5 mg/kg of morphine in naive animals was nearly equivalent to that of 10 mg/kg of the drug in the animals treated with 2.5 mg/kg of reserpine. Daily administration of 5 mg/kg of morphine developed tolerance rapidly, and at the 4th injection, this dose of morphine did not produce any analgesia (Fig. 2).

b) Effect of repeated small dose of reserpine: Daily repeated injection of a small dose of reserpine, 0.1 mg/kg/day, did not modify the brain level of norepinephrine and dopamine for 10 days; however, serotonin content was decreased significantly by the treatment. In these animals, the analgesic effect of morphine was not altered from the effect in control animals during 10 days treatment. A single dose of 0.1 mg/kg of reserpine failed to maintain the analgesic effect of morphine except on the 2nd day, and

![Fig. 2. Analgesic effect of morphine and development of tolerance after a single dose of reserpine. Animals were treated with a single dose of 2.5 mg/kg of reserpine, i.p.. Starting from 1, 5 and 10 days after reserpine treatment, the analgesic effect of morphine, 10 mg/kg, i.p., was measured daily for 6 days. The analgesic effect of morphine at the day of the start of injection is shown by closed symbols (■, △, ○). Control animals were treated with saline instead of reserpine; then 24 hr after the treatment morphine injection, 10 (×) or 5 (×) mg/kg was started. The analgesic effect (the response time, a cut-off time of 6 sec) was measured by the modified Haffner's method. The effect was calculated by plotting the increase of response time (sec) against time (min), and the AUC was expressed as a percent of the effect of 10 mg/kg of morphine obtained in naive control animals. #, ##: Significantly different from the effect in naive animals (P<0.05, P<0.01). *, **: Significantly different from the effect of morphine at the start of daily injection of the corresponding group (P<0.05, P<0.01).]
Fig. 3. Changes in brain level of monoamines and development of tolerance in chronically reserpinized mice. Animals were daily treated with 0.1 mg/kg of reserpine i.p., for 10 days, and brain level of monoamines was determined at the day indicated in the Figure. To estimate the development of tolerance, 24 hr after the initial injection of reserpine, daily administration of 10 mg/kg of morphine was started. In these animals (●), reserpine was daily administered after every measurement of the analgesic effect. The other group of animals was treated with a single injection of 0.1 mg/kg of reserpine (○), and the development of tolerance was estimated as above. For other details, refer to the footnotes of Figs. 1 and 2.

Discussion

Accumulating evidence suggests the important role of brain catecholamines in morphine analgesia (1–6) and the development of tolerance to the effect (7). Among the reports, Takagi et al. (1, 5, 6) have described that tetrabenazine, a synthetic analogue having little peripheral effect, antagonized the analgesic effect of morphine in mice, and the effect of tetrabenazine may be attributed to a reduction of brain dopamine and norepinephrine. Takagi and Kuriki (7) have also reported that tetrabenazine given 2 hr before daily injection of morphine markedly suppressed the development of tolerance, and the suppressive effect was antagonized by repeated administration of DOPA. Based on these findings, they suggested that brain catecholamines, rather than serotonin, might be involved in the development of tolerance to morphine. Actually, in the present experiment, the analgesic effect of morphine was significantly reduced after a single large dose of reserpine in parallel with the reduction of brain monoamine content. In these animals, the analgesic effect of morphine did not change during 5 daily repetitions, and the development of tolerance was suppressed. The analgesic effect of 5 mg/kg of morphine in naive mice was nearly equivalent to that of 10 mg/kg of the drug in the animals treated with 2.5 mg/kg of reserpine on the previous day. Daily administration of 5 mg/kg of morphine produced tolerance, so that the suppression of the development of tolerance in reserpinized animals is not due to the lowered effect of morphine. Although the brain level of monoamines and morphine analgesia had not returned to the control level, when daily injection of morphine was started from 10 days after reserpine treatment, tolerance developed as rapidly as in the control animals. On the other hand, a low dose of reserpine which did not induce any appreciable changes in brain catecholamine content could effectively block the development of tolerance to morphine. These results are fundamentally different from the previous report (7) that the decrease in brain catecholamine content is responsible for the suppression of morphine
tolerance and clearly indicate that the suppression of the development of tolerance to morphine analgesia is not attributed to the reduction of brain norepinephrine and dopamine by reserpine. When animals were daily treated with 0.1 mg/kg of reserpine, the serotonin content in the brain was significantly reduced. Thus, the possible involvement of the serotonergic mechanism in the suppressive effect of reserpine for the development of tolerance cannot be excluded. However, in our preliminary experiment, we found that intracerebroventricular injection of 6-hydroxydopamine selectively lowered the brain content of norepinephrine without any changes in dopamine and serotonin levels, and this treatment also effectively suppressed the development of tolerance to morphine analgesia (data not shown).

We have previously provided an evidence that complete masking of morphine analgesia by naloxone could not prevent the development of tolerance, and suggested the possibility of dissociating the analgesic effect from tolerance liability (8). Here, we present another evidence that the analgesic effect of morphine is not altered by repeated administration, and the development of tolerance is completely suppressed in reserpinized animals. This fact may support our previous finding that morphine analgesia and tolerance are separable from each other by underlying mechanisms.

References