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Stimulation-Produced Analgesia Under Repeated Morphine Treatment in Rats

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MOROZOVA, A. S. AND E. E. ZVARTAU. *Stimulation-produced analgesia under repeated morphine treatment in rats.* PHARMACOL BIOCHEM BEHAV **25**(3) 533–536, 1986.—Components of complex emotional reaction to nociceptive stimulation as well as antinociceptive effect of periaqueductal gray matter (PAG) electrical stimulation were determined in rats. Animals were treated with morphine hydrochloride or saline for 10 days. Morphine analgesic effect during subchronic dosage (50 mg/kg a day) was gradually decreased. The same was true for stimulation-produced analgesia (SPA). Naloxone (2 mg/kg) exerted a partially antagonistic effect in relation to SPA in saline-treated and failed to abolish SPA in morphine-treated rats. It is concluded that the opiate component of the antinociceptive system is of importance for the tolerance development to morphine-like drugs.

Morphine Naloxone Stimulation-produced analgesia Opiate tolerance Antinociceptive system

STUDIES of the past decade have demonstrated an important role of the antinociceptive brain system in the modulation of the afferent flow and in the mechanism of the action of narcotic analgesic drugs [3, 8, 11, 13]. Endogenous opioids are involved in the realization of stimulation-produced analgesia (SPA), and intensive stimulation of the periaqueductal grey matter (PAG) may decrease the antinociceptive potency of morphine [6,7]. Therefore, changes in the activity of the antinociceptive system might be of importance in neurophysiological mechanisms responsible for the phenomenon of opiate tolerance.

The present study examined the effect of subchronic morphine hydrochloride treatment on the structure of the complex pain reaction and the effect of SPA in rats. Previous work from this laboratory has shown the important role of emotional mechanisms in the phenomena of tolerance and dependence [12]. Therefore, emphasis is now placed on the analysis of the structure of responses to nociceptive stimuli with special reference to affective components.

METHOD

Subjects

The subjects were 10 drug naive male rats, 250–300 g body weight. They were housed in groups of 2–3 in plastic cages. Food and water were provided ad lib.

Antinociceptive Effect Determination

The animals were implanted with electrodes in PAG according to stereotaxic coordinates of the König and Klippel atlas [4]. The electrodes of nichrome wires 150–200 μ in diameter were insulated except at the tip. All surgery was performed under pentobarbital (40 mg/kg) anaesthesia.

Antinociceptive effect was produced by applying PAG electrical stimulation with the following parameters: frequency 50–100 imp/sec, pulse duration 0.1 msec, amplitude I–10 V. The pain reaction was determined by electrical stimulation of the tail. The method has been described in detail elsewhere [9]. Briefly, concentric electrodes were fixed around the tail. Stimulation (6–8 imp/sec, pulse duration 0.2–0.5 msec) was gradually increased during a 20 sec period. Thresholds of the following components of the complex behavioral reaction were recorded: tension of the tail, startle reaction and increase in respiration frequency (usually occuring simultaneously), initial paw and body movements, turn of the head toward electrodes, squeak, touching the electrodes, biting the electrodes, circling and flight, squealing, gnawing the electrodes. The intensity of stimulation sufficient to produce the initial pattern (tail tension, startle, tachipnoe) was defined as conventional "threshold unit." Generalized emotional pain reaction was usually observed with 5–6 fold increase in "threshold" stimulation.

Three individual intensities of PAG stimulation were adjusted for every animal. "Threshold stimulation" (TS) eliminated some manifestations of the emotional pain reaction. "Minimal suprathreshold stimulation" (STS_{min}) resulted in the suppression of most emotional components, while "maximal suprathreshold stimulation" (STS_{max}) evoked total inhibition of the nociceptive response. In every trial, the nociceptive stimulation of the tail was applied 5 sec after the onset of PAG stimulation.

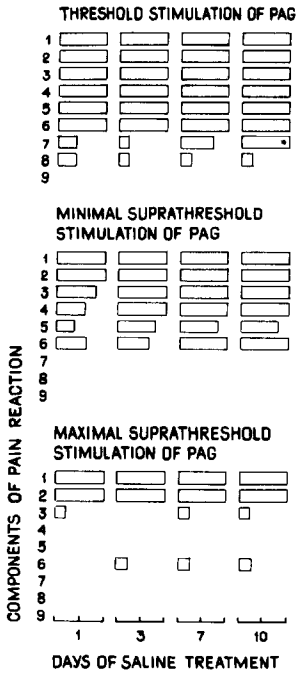

FIG. 1. The change of stimulation-produced analgesia during repeated saline administration. Components of the pain reaction: tail tension, startle, tachypnoe (1), paws and body movements (2), turn of the head to electrodes (3), touching the electrodes (4), biting the electrodes (5), squeak (6), circling and flight (7), squealing (8), gnawing the electrodes (9). The size of the column reflects the frequency of component's appearance in %%. *-statistically significant ($p<0.05$, Fisher test) comparing with day 1.

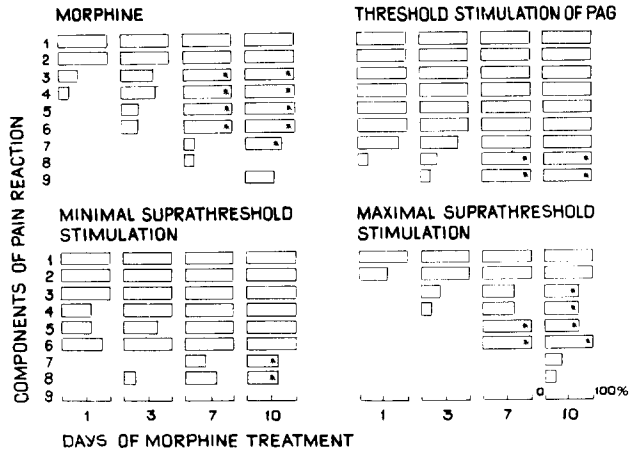

FIG. 2. The change of the structure of the pain reaction in morphine-treated rats. Morphine was administered IP twice daily (12 mg/kg at 9–10 a.m. and 38 mg/kg p.m.) during 10 days. Left top panel illustrates the development of tolerance to analgesic effect of morphine tested 30 min after the administration of the drug. Other panels demonstrates the tolerance to stimulation-produced analgesia tested 4 hr after morphine administration. See Fig. 1 for other details.

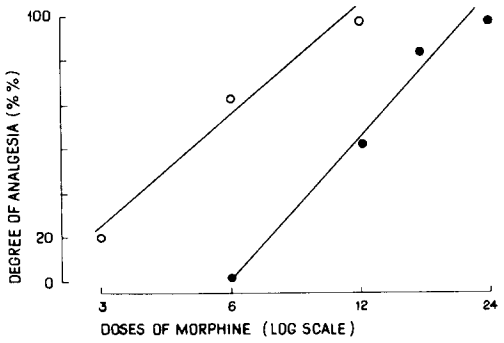

FIG. 3. The relation between the dose and analgesic effect of morphine before (○—○) and after (●—●) subchronic administration of the drug. The degree of analgesia was estimated in the group of rats as percent of trials without such emotional components of pain reaction as gnawing, flight and squealing. Under the control conditions these manifestations were observed in all trials.

Experimental Protocol

Group 1 rats (n=5) were administered morphine hydrochloride IP twice daily (12 mg/kg at 9–10 a.m. and 38 mg/kg at 5–6 p.m.) for 10 days. Before and after the cycle of injections, the relation between the dose and analgesic response was estimated. Antinociceptive action of morphine was determined 30 min and SPA 4 hr after the administration of the drug. SPA was tested also before "morning dose" of morphine (14–17 hr after the previous "evening dose"). On day 10 naloxone hydrochloride (Endo Lab) was administered IP in the dose of 2 mg/kg 30 min after morphine injection. SPA was tested before and 15 min after naloxone administration.

Group 2 rats (n=5) were studied according to the same schedule with isotonic saline (0.1 ml/100 g weight) being administered instead of morphine.

Histological examination of the brains of experimental animals confirmed the location of electrodes in PAG area.

RESULTS

In the control group of rats, no significant tolerance was observed to the antinociceptive effect of PAG stimulation (Fig. 1). Only one component of the complex pain reaction under TS was restored by day 10 of the experiment, while the general structure of the reaction after STS remained unaffected.

Morphine in the dose of 12 mg/kg abolished a number of components of the complex pain reaction (Fig. 2). Further administration of morphine decreased the analgesic effect. On day 10 the drug suppressed only a few affective manifestations. Determination of the dose-response relations revealed a shift of the regression line to the right (Fig. 3). ED_{50} of the suppression of the affective pain response was 5 mg/kg before and 12.6 mg/kg after the course of morphine injections.

Besides the development of the tolerance to morphine's analgesic action, characteristic changes were observed in the spontaneous behavior of rats before "morning dose" (15–17 hr after previous injection). Ptosis and piloerection, depression of orienting and searching behavior appeared on days 3–4. Later (days 7–8) the reaction to handling became stronger. The tolerance to morphine-produced analgesia developed simultaneously with an increase in the sensitivity to pain stimulation (Fig. 4). Naloxone injected at the peak of morphine effect on day 10 completely blocked analgesic action and provoked such withdrawal signs as jumping, grooming, head shakes and hyperreactivity to handling.

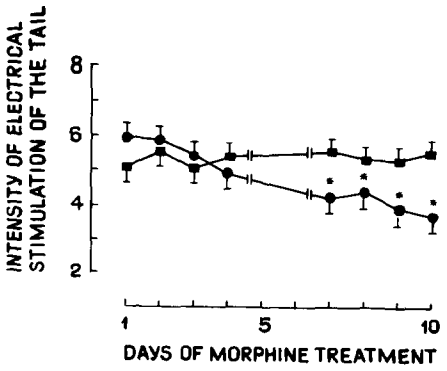

FIG. 4. The change of thresholds of the generalised pain reaction under subchronic administration of morphine (●—●) and saline (■—■). Generalised pain reaction was tested before the "morning dose" of morphine (14–17 hr after the previous administration of the drug). Intensity of stimulation is expressed in conventional threshold units. One threshold unit is the intensity of the electrical stimulation needed to evoke initial response (tail tension, startle and tachypnoe). Generalised reaction was usually observed in 5–6-fold increase of the intensity of the threshold stimulation. *Statistically significant ($p<0.05$, Wilcoxon t-test) compared with day 1 of the experimental group.

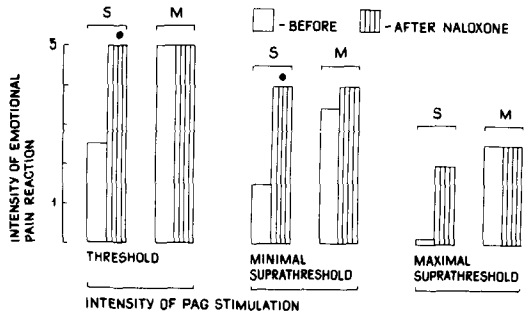

FIG. 5. The influence of naloxone on stimulation-produced analgesia after subchronic administration of morphine and saline. Intensity of the emotional pain reaction is expressed as a sum of probabilities of appearance of such symptoms as biting the electrodes, squeak, circling and flight, squealing, gnawing the electrodes. Maximal reaction corresponds to 5 points. S—saline, M—morphine-treated groups. On day 10 animals were administered morphine (12 mg/kg) or saline. In 30 min naloxone (2 mg/kg) was injected. SPA was tested before and 15 min after naloxone administration. ●—significant as compared to data before naloxone injection ($p<0.05$. Fisher test).

Repeated morphine administration affected SPA significantly. The strongest inhibition of SPA up to its complete disappearance was observed under TS of the PAG (Fig. 2, right top panel). On days 3–4 the number of animals demonstrating such symptoms as gnawing the electrodes and squealing increased. On day 7, TS of PAG failed to produce the antinociceptive effect.

The changes in the pain response structure under STS developed more slowly. Only on day 7 was SPA significantly diminished. On day 10 this effect became significant for a number of manifestations (Fig. 2, bottom panels). A similar time-course of SPA changes was noted 4 and 16 hr after morphine administration.

In the control group, naloxone on day 10 completely blocked SPA produced by TS and exerted a partially antagonizing action under both suprathresholds stimulations (Fig. 5). In morphine-tolerant rats with lower SPA, naloxone failed to antagonise the antinociceptive effect of PAG electrical stimulation (Fig. 5).

DISCUSSION

The development of tolerance to the analgesic effect of morphine is well documented in the literature (see [5] for review). Our experiments revealed a distinct opiate antinociceptive action—SPA-cross-tolerance. Long-lasting PAG stimulation is known to diminish morphine's analgesic effect [6,7]. The intensity of PAG stimulation in the present study was significantly weaker and the tolerance to SPA failed to develop in saline-treated controls. Thus, the diminution of SPA appears to be the result of chronic opiate stimulation and suggests the involvement of brain stem antinociceptive systems in the mechanisms of tolerance to morphine's analgesic action.

Morphine's analgesic effect was completely abolished by naloxone in the dose of 2 mg/kg. In the control group, naloxone showed an antagonistic action to SPA. However this antagonism might be characterised as partial, especially in relation to the stronger stimulation of PAG. These results confirm previous data [1, 14, 15] and suggest the existence of both opiate and non-opiate mechanisms of SPA. In morphine-tolerant animals, naloxone failed to affect SPA. These data are indicative of the predominant changes in the "opiate component" of the SPA during chronic opiate stimulation.

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