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Reserpine-induced Changes in Anesthetic Action of Fentanyl

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The effect of prior administration of reserpine on fentanyl dose–response curves for loss of the righting reflex and prevention of purposeful movement response to noxious stimulation was studied in rats. It was found that reserpine (5 mg·kg^{-1}, 3 h before the tests) antagonized the effect of fentanyl on purposeful movement response to the tail clamp and, at the same time, strengthened its effect on the righting reflex. As a result, reserpine pretreatment reversed the order of fentanyl potency regarding these two effects. Reserpine changed fentanyl ED_{50} values for the purposeful movement response from 8.2 to 20.3 $\mu g \cdot kg^{-1}$ ($P < 0.0001$) and for the righting reflex from 20.5 to 13.7 $\mu g \cdot kg^{-1}$ ($P < 0.0001$). The results suggest that reserpine dissociates the analgesic action of fentanyl from its anesthetic action, defined as a loss of the righting reflex. This may be regarded as an indication that the analgesic action of narcotics may not adequately reflect their anesthetic potency. (Key words: Analgesics: fentanyl. Anesthetics, intravenous: fentanyl. Ataractics: Rauwolfia, reserpine. Potency, anesthetic: ED_{50}. Righting reflex.)

THE USE OF HIGH DOSES of narcotic analgesics to achieve desired anesthetic objectives is becoming a current practice in anesthesia. Some narcotic analgesics are now used as sole (complete) intravenous anesthetics. Stanley has classified these agents as intravenous narcotic anesthetics.[1] Evaluation of narcotics suggested for anesthesia usually is performed with methods developed for the assessment of antinociceptive action of opiates. It is well known that general anesthesia includes several components.[2] If the strength of action of narcotics is related equally to all components of anesthesia, the analgesic potency of narcotics adequately may reflect their anesthetic potency. However, there is evidence suggesting the opposite.

The comparison of morphine and fentanyl regarding their ability to prevent purposeful movement (PM) response to the tail clamp and to abolish the righting reflex (RR) has shown that potency ratios of these agents are different for the studied end points. The ratios of RR ED_{50} to PM ED_{50} were 7.8 for morphine *versus* 2.4 for fentanyl.[3] Those results suggested that the relationship between the strength of action of the narcotic analgesics on movement response to noxious stimulation (a common index for analgesic action of opiates) and on the righting reflex (a classical index of anesthesia for intravenous anesthetics) may be different for different opiates. Additionally, not only opiates but also opioid peptides induce anesthesia defined as loss of the RR in rodents.[4]

In this study, we tried to provide data to answer the question of whether the analgesic action of fentanyl adequately reflects its anesthetic potency, defined as loss of the righting reflex. Accordingly, we made an attempt with the use of reserpine to dissociate the analgesic action of fentanyl (prevention of purposeful movement to tail clamp) from its anesthetic action (loss of the righting reflex). We have chosen reserpine because it antagonizes opiate-induced analgesia.[5,6] At the same time, it was reported that reserpine decreased halothane anesthetic requirements (it strengthened the effect of halothane on movement response to the tail clamp in rats).[7]

Methods

The experiments were done on 110 male Sprague–Dawley rats weighing 275–325 g. Effects of fentanyl were determined using the following criteria: 1) loss of the righting reflex; the test was regarded as positive if the rat failed to right itself (with all four feet on the table) within 15 s after being placed in a side position; and 2) prevention of purposeful movement response to a noxious stimulus; the animals were stimulated for 60 s by placement of a hemostat on the middle of the tail (pressure of 300 g/mm^2). Purposeful movement toward the clamp was considered a positive response to the stimulation. Because the movement often was finished with clamp biting, the method was very similar to Haffner's method.[8]

With each of the two end points, two series of experiments were performed, one in animals injected with reserpine (Ciba)—5 mg/kg, ip, 3 h before a test—and another in control animals (saline). The dose of reserpine and time interval have been found adequate for depletion of monoamines in the central nervous system (CNS). Fentanyl citrate (Janssen) was injected into the saphenous vein over 10 s. The end points were determined 5 min after injection of fentanyl, which corresponds to its peak effect[3,10] and time of its maximal level in cerebrospinal fluid (CSF).[11,12] Every animal

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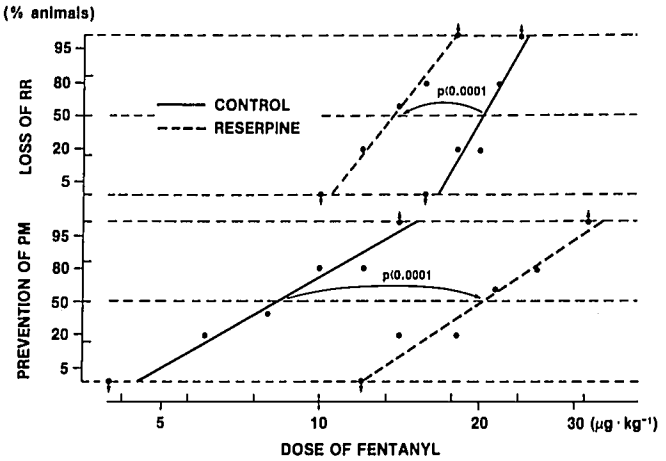

FIG. 1. Reserpine-induced changes in fentanyl dose–effect curves for the righting reflex (RR) and purposeful movement (PM) response to noxious stimulation. Along the vertical axis, the percentage of animals (on a probit scale) that reached the specified endpoints. Along the horizontal axis, doses of fentanyl (on a log scale). Each dot represents a group of five animals at the indicated dosage.

received only one predetermined dose of fentanyl, and, following this, the presence or absence of an end point was determined only once. The rats were kept in a Plexiglass® chamber where they breathed pure oxygen.

In all four series of experiments, fentanyl dose–effect curves were determined. We gave the same dose to five rats and studied five doses in the righting reflex series and six doses in the purposeful movement response series. In one group of animals, the dose of the drug was low enough so that all animals were unaffected. In another group, it was high enough so that all were affected. Dose levels for these two groups were determined in preliminary experiments. In the remaining groups, the doses of the drug were spaced between the above mentioned marginal doses. As a result, the following doses of fentanyl were used: the RR series ($\mu g \cdot kg^{-1}$): 16, 18, 20, 22, and 24 in control rats, and 10, 12, 14, 16, and 18 in reserpine pretreated rats; in the PM series ($\mu g \cdot kg^{-1}$): 4, 6, 8, 10, 12, and 14 in control rats, and 12, 14, 18, 22, 26, and 32 in the pretreated rats. Volume of the injection was 0.3–0.5 ml.

Dose–effect curves were calculated, and fentanyl ED_{50} values for the righting reflex and the purposeful movement response with and without reserpine pretreatment were derived. All statistical validations were performed with the use of probit analysis.[13] Animal care standards in this study were in accordance with federal and institutional policy and standards of the American Association for Accreditation of Laboratory Animal Care as specified in the Guide for the Care and Use of Laboratory Animals.‡

Results

Figure 1 shows fentanyl dose–effect curves for loss of the righting reflex and prevention of purposeful movement with and without reserpine pretreatment. The central points of the curves represent the median effective doses (ED_{50}). In numeric form, they are presented in table 1. ED_{50} values for the righting reflex in the control group are greater than those for the purposeful movement response, which is typical for opiates. According to figure 1 and table 1, reserpine weakened the effect of fentanyl on the movement response and, on the contrary, enhanced the effect of fentanyl on the righting reflex. After reserpine, the dose–effect curve for the righting reflex shifted to the left along the dose axis, and the dose–effect curve for the movement response shifted to the right. As a result, fentanyl ED_{50} for the righting reflex was decreased from 20.5 to 13.7 $\mu g \cdot kg^{-1}$ ($P < 0.0001$) and ED_{50} for the movement response—from 8.2 to 20.3 $\mu g \cdot kg^{-1}$ ($P < 0.0001$).

Another interesting observation is the difference in the slopes of the dose–effect curves for the righting

‡ DHEW Publication No. (NIH)78-23: Guide for the Care and Use of Laboratory Animals. Washington, D. C., U. S. Government Printing Office, 1978.

TABLE 1. Reserpine-Induced Changes in Anesthetic Action of Fentanyl

	Fentanyl ED_{50} ($\mu g \cdot kg^{-1}$)		Slope of Fentanyl Dose–Response Curve	
	Control	Reserpine*	Control	Reserpine*
Loss of RR	20.5 (19.3–22.3)†	13.7 (11.9–15.3) $P < 0.0001$	24.7 ± 7.8‡	16.6 ± 5.6
Prevention of PM	8.2 (6.3–10.0)	20.3 (16.9–24.6) $P < 0.0001$	7.5 ± 2.3 $P < 0.05$§	8.6 ± 2.6

RR = righting reflex; PM = purposeful movement response to noxious stimulation.
* 5 $mg \cdot kg^{-1}$, iv, 3 h before the test.
† Ninety-five per cent fiducial limits.
‡ Standard error.
§ As compared with RR slope.

reflex and for purposeful movement response, which was statistically significant in the control groups of animals: 24.7 *versus* 7.5, $P < 0.05$ (table 1). The slopes of the dose–effect curves in the control *versus* the reserpine pretreated animals, in both the RR series and in the PM series, did not demonstrate any statistically significant difference.

Discussion

Both loss of the righting reflex[14] and prevention of the movement response to noxious stimulation[15] are classical indices of anesthetic action for inhalational and intravenous anesthetics. With narcotic analgesics, the movement response to noxious stimulation is difficult to regard as an index of anesthesia because this response disappears at the time when animals continue to demonstrate behavior of conscious animals. Therefore, the abolition of movement response by narcotics probably could be regarded as nothing more than a sign of analgesia.

In our experiments, reserpine enhanced the effect of fentanyl on the righting reflex and weakened its effect on the movement response to noxious stimulation. As a result, reserpine pretreatment reversed the order of fentanyl potency regarding the righting reflex and movement response to noxious stimulation. With opiates, blockade of purposeful movement to noxious stimulation invariably occurs at doses lower than those required to block the righting reflex. After reserpine, the order of fentanyl potency regarding these two effects resembles that of intravenous nonnarcotic anesthetics. Comparison of the position of RR and PM dose–effect curves for etomidate[16] with those for fentanyl after reserpine pretreatment shows an unusual similarity. After reserpine, at a dose level of fentanyl that blocks the righting reflex in 95% of animals, about 75% of them are still capable of completing purposeful movement in response to tail clamping; at a dose level of etomidate that blocks the righting reflex in 95% of animals, about 85% of them are still capable of movement to tail clamping.

If anesthetic potency of fentanyl is defined as loss of the righting reflex, our data show a dissociation between the indices of analgesic and anesthetic action. This observation suggests that the analgesic potency of opiates may not reflect their anesthetic potency. Such a suggestion agrees well with our previous study[3] which showed that the ratios of RR ED_{50} to PM ED_{50} were 7.8 for morphine *versus* 2.4 for fentanyl, $P < 0.001$ (slopes of the dose–effect curves were not tested for parallelism).

A number of publications indicate that reserpine antagonizes morphine analgesia.[5,6,17] Our data on antagonism between reserpine and fentanyl are in accordance with these publications. As far as reserpine–morphine interaction for the righting reflex is concerned, no information on this subject can be found in the literature. There are, however, data on halothane–reserpine anesthetic interaction. Miller *et al.*[7] have shown that reserpine, as well as guanetidine, significantly strengthen the effect of halothane on movement response to the tail clamp in rats. If motor response to noxious stimulation reflects a halothane-induced anesthetic process in the same way as the righting reflex reflects a fentanyl-induced anesthetic process, then strengthening of fentanyl anesthetic action after reserpine, which was observed in our experiments, is not an unexpected change.

It was found that opioid peptides also have an anesthetic action defined as loss of the righting reflex.[4] Dodson and Miller[18] reported that anesthetic action (loss of RR) of a leucine enkephalin analog was reversed with pressure, and its anesthetic potency correlated with its lipid solubility (according to the Meyer–Overton type of action). The authors suggested that there are two mechanisms of anesthetic action for opioids: opiate receptor specific mechanism and nonspecific mechanism related to lipid solubility.

It was found in this study that the slopes of the fentanyl dose–effect curves for the righting reflex and for the movement response are different. Parallel dose–effect curves are indicative of the identity of the mechanism of action.[19] The absence of parallelism, therefore, suggests different mechanisms through which fentanyl produces its analgesic and anesthetic effects. Thus, three pieces of evidence exist that suggest that mechanisms underlying opiate-induced loss of the righting reflex and prevention of movement response to noxious stimulation are different: 1) reserpine-induced dissociation of the effects of fentanyl on these two indices; 2) difference in the slopes of the appropriate fentanyl dose–effect curves; and 3) difference in the ratios of RR ED_{50} to PM ED_{50} for morphine and fentanyl. Our results do not provide information on what specific mechanisms underlie this difference (opiate *vs.* nonopiate action, one subtype of opiate receptor *vs.* another subtype, *etc.*).

We conclude that reserpine dissociates the analgesic action of fentanyl from its anesthetic action, defined as a loss of the righting reflex. This may be regarded as additional evidence, indicating that the analgesic action of narcotics may not adequately reflect their anesthetic potency.

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