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Characterization of the Acute Tolerance and Dependence to Morphine-Induced Respiratory Depression in Decerebrate Cats [1]

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Acute tolerance to the respiratory depression was produced in decerebrate cats by injecting six consecutive doses of morphine, 2 mg/kg each, at one hour interval. The course of tolerance was examined independently for frequency and tidal volume, in resting as well as in CO_2-stimulated breathing.

Regarding respiratory frequency, maximal depression was induced by the first dose of morphine; subsequent doses did not depress frequency any further. Recovery toward control level was initiated after the second dose. Maximal tidal volume depression was observed after two or three doses of morphine; the values remained at the lowest level for the rest of the experiment. Cross-tolerance between morphine and fentanyl was readily obtained, for frequency as well as for tidal volume. Pentobarbital did not show cross-tolerance with the analgetics.

Acute dependence was unmasked by naloxone, 1 mg/kg. Reversion of the depression and overshoot were observed, especially on the part of respiratory frequency. Naloxone shifted to the left of the control curve and increased the slope of the CO_2-ventilation relationship. This effect demonstrates the hyper-excitability created on the respiratory center during the development of dependence.

In recent years several models have been designed to analyze the mechanisms implied in the production of acute tolerance to and dependence on morphine. Most of the studies include opioid effects related to nociceptive reactions and motor activity in rodents (1, 13). Little attention, however, has been paid to the respiratory depression induced by narcotics, in spite of the fact that acute tolerance was produced following a short-term infusion of morphine in awake dogs (7), and acute dependence was unmasked by nalorphine in decerebrate cats subjected to a single

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injection of morphine (8). In a recent study performed in decerebrate cats (2), one dose of morphine was also able to produce tolerance to the depressant effect of morphine on the respiratory frequency.

The respiratory function has been shown to serve as an adequate model for studying the influence of the neuroamines upon the acute effects of morphine on the central nervous system (3). Since the central amines are considered as potential modulators of the development of tolerance and dependence to the narcotic analgetics (4), an investigation was undertaken to determine their influence on the production of these two syndromes, as judged by the course of the morphine-induced respiratory depression. The present communication reports the features of the tolerance to the respiratory depressant effects of morphine, produced by frequent injections of the drug in decerebrate cats. Furthermore, the respiratory characteristics observed, following the unmasking by naloxone of the acute physical dependence, will be presented.

Materials and Methods

Experiments were performed on 35 cats of both sexes, weighing between 2.0 and 3.5 kg. Decerebration of the *cerveau isolé* type was prepared under halothane anesthesia as previously described (3). Respiration was recorded by means of a plastic body plethysmograph through a Harvard Respiration recording module. The plethysmograph was calibrated at the end of each experiment. Blood pressure was recorded from a carotid artery and measured with a Harvard transducer and electronic module. All records were made on a Harvard Chart Mover. End-tidal CO_2 concentration was monitored by continuous sampling of tracheal air through a Godart CO_2 infrared analyzer, and was recorded on a Godart Omniascriptor. Rectal temperature was maintained at $38 \pm 0.5°$ C. The animals were given O_2 to breath throughout the experiment.

The respiratory function was estimated by measuring: 1) the spontaneous resting respiratory parameters, and 2) the respiratory response to the stimulation with CO_2 5 % in O_2, administered by excess flow around the tracheal cannula over a period of 5 min.

Experimental design. Observations were not begun until at least 2 hr following discontinuation of halothane. A group of 12 cats was given six injections of morphine in the cephalic vein, at one hr interval, the dose of each injection being 2 mg/kg. Resting respiration was evaluated 5, 15 and 60 min after injection. The respiratory response to CO_2 was observed 15 min after the administration of the narcotic. One hr after the last dose of morphine, naloxone was injected at the dose of 1 mg/kg i.v., and its effect was followed up for 60 min. The CO_2 response was observed 15 min after the injection. Fentanyl, 0.02 mg/kg i.v., was injected as an initial narcotic into 5 cats. For cross-tolerance studies, two groups of 5 cats each were used. In the first group, fentanyl was given after the fourth dose of morphine, and in the second, it was given after the sixth dose of morphine. In a few morphinized animals, sodium pentobarbital, 20 mg/kg i.v., was randomly administered.

Drugs. The following drugs were used: Morphine hydrochloride (Miró); naloxone hydrochloride (Endo Laboratories); fentanyl (fentanest®, Latino); sodium pentobarbital (nembutal®, Abbott), and halothane (fluothane®, ICI-Farma).

Results

Morphine tolerance. The first dose of morphine (Fig. 1, M_1) decreased the resting respiratory frequency, tidal volume and minute volume; a steady level of de-

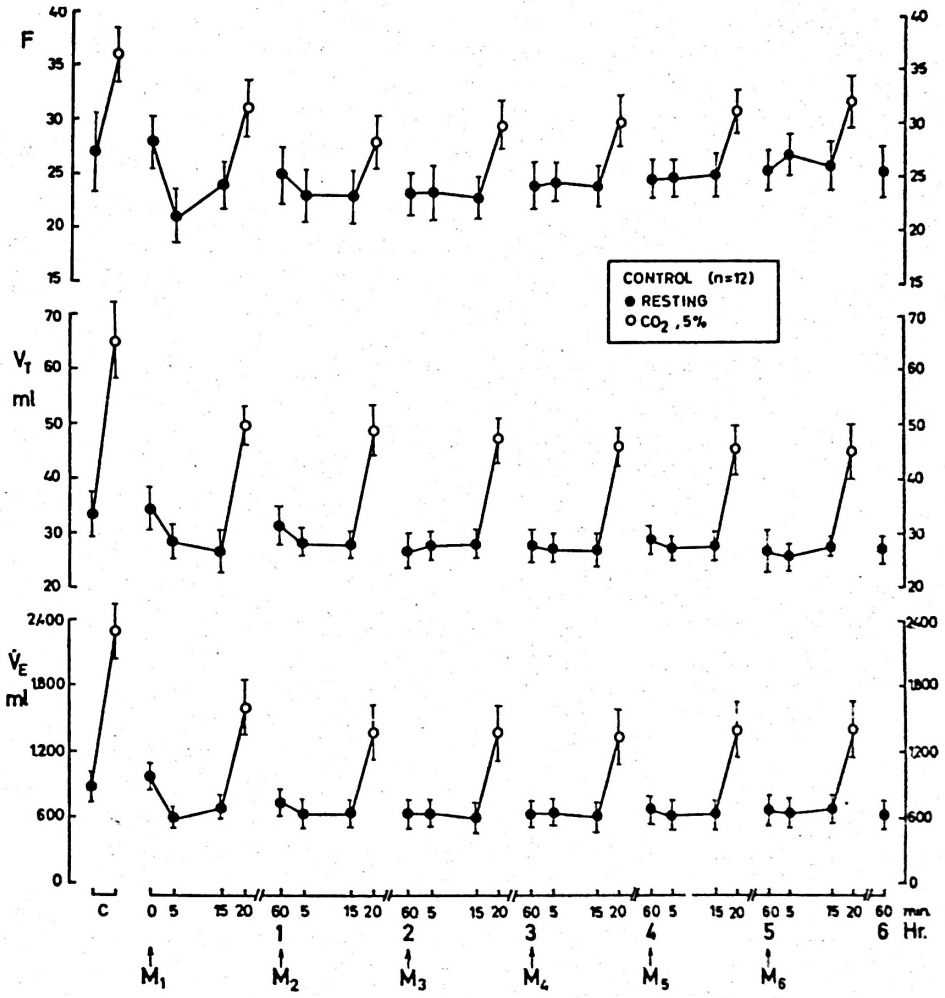

Fig. 1. *Effects of six doses of morphine, 2 mg/kg each (M_1 to M_6), upon the respiratory frequency, tidal volume and minute volume in decerebrate cats.*
Black symbols represent respiratory values during resting respiration, open symbols represent values obtained after stimulation with 5 % CO_2 in O_2. C = control period. Values plotted represent mean ± S.E.M.

pression was attained in about 15 min. At this time, frequency and tidal volume responses to CO_2 were depressed, and remained constant during the whole hour. The second and third doses of morphine (M_2 and M_3) produced further depression of the resting tidal and minute volumes, and of their responses to CO_2. On the other hand, little or none effect on frequency was observed. The three subsequent doses of morphine (M_4-M_6) did not induce any further depression of respiration. A steady reversion of the resting frequency and of its response to CO_2 toward control values consistently appeared between M_2 and M_3. On the other hand, tidal volume values remained at the lowest level throughout the experiment. The values of minute volume were consistent with the changes induced on the other two parameters, so that a slight recovery could be observed between M_4 and M_6.

Effect of naloxone. The injection of the narcotic antagonist naloxone abruptly antagonized the respiratory depression and induced a sharp increase in heart rate and blood pressure. Resting frequency and tidal volume were increased inmediately, and end-tidal CO_2 levels dropped considerably. Naloxone reverted the depression of all the respiratory parameters beyond control values (Fig. 2). However, the resting frequency was brought to a much higher level than resting tidal volume. Similarly, the response of frequency to CO_2 clearly exceeded that obtained during the control period, whereas the CO_2 response of tidal volume only reached the control values.

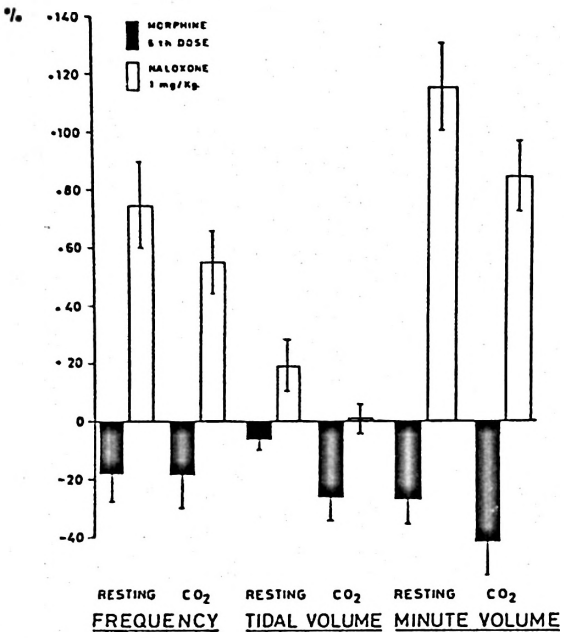

Fig. 2. *Antagonistic effects of naloxone upon the morphine-induced depression on respiration.*
Bars represent mean vaules ± S.E.M. of the percent change related to the pre-morphine control period.

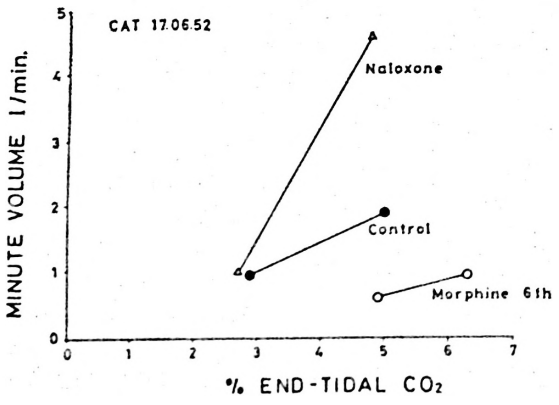

Fig. 3. *Effects of the sixth dose of morphine and of naloxone upon the steady-state relationship between minute volume and end-expiratory CO_2 in a decerebrate cat.*

The responsiveness of the respiratory center to CO_2 was also restored to a level higher than the control (Fig. 3). Not only the relationship between minute volume and end-tidal CO_2 was shifted to the left of the control curve, but the slope was markedly increased.

Cross-tolerance studies. The respiratory depression induced by fentanyl was characterized by a short latency, the peak effect being attained in about 5 min (Table I). Differences in latency and potency between fentanyl and morphine seem to be accounted for by differences in their lipophilic properties (5). Considerable reversion of the depression was observed at 15 min. Since the response to CO_2 was measured at this time, the values of the responses to CO_2 do not reflect the full action of the drug, nor can they be compared with the values of the morphine series. However, they are valuable for comparative purposes between non-morphinized and morphinized cats subjected to the treatment of fentanyl. Fentanyl was significantly less active in the two groups of morphinized cats than in the non-treated cats, for every respiratory parameter. No significant difference was observed between the effect of fentanyl in the group pretreated with 4 doses of morphine and the group treated with 6.

Effects of pentobarbital. Equidepressant doses of pentobarbital were injected

Table I. *Respiratory effects of Fentanyl, 0.02 mg/kg, in control decerebrate cats and in decerebrate cats pretreated with 4 and 6 doses of morphine, 2 mg/kg.*

Values are expressed as percent change related to the control period ± S.E.M.

	CONTROL (n = 5)			MORPHINE PRE-TREATMENT					
				4 × 2 mg/kg (n = 5)			6 × 2 mg/kg (n = 5)		
	5 min	15 min	CO_2 5 %	5 min	15 min	CO_2 5 %	5 min	15 min	CO_2 5 %
Frequency	—44.7±5.4	— 7.5±2.1	— 9.9±2.0	— 5.8±1.1	— 8.8±5.0	+0.1±4.0	—5.4±2.7	+1.4±3.4	—8.8±10.7
Tidal volume	—28.0±3.3	— 7.3±1.5	— 9.8±2.0	— 8.5±4.8	— 5.5±4.0	+6.0±8.5	—1.1±1.0	+5.4±3.2	+3.2±2.5
Minute volume	—58.0±5.6	—14.0±2.9	—18.0±2.3	—15.2±3.2	—14.2±4.8	+4.0±8.1	—9.8±3.9	+8.2±7.4	—2.2±8.5

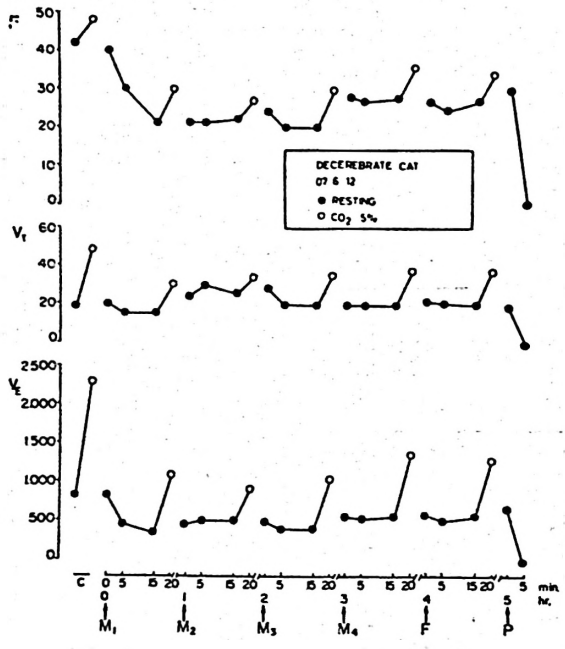

Fig. 4. *Effects of four doses of morphine, 2 mg/each (M_1 to M_4), upon the respiratory frequency, tidal volume and minute volume in a decerebrate cat, followed by one dose of fentanyl (F), 0.02 mg/kg, and one dose of pentobarbital (P), 20 mg/kg.*

into 3 cats at 1 hr interval. Progressive respiratory depression was obtained in all animals, so that respiratory failure appeared after the third dose. In 5 preparations made tolerant to morphine and fentanyl, one single dose of pentobarbital was sufficient to kill the animal. A characteristic example is shown in Fig. 4.

Discussion

The present results indicate that the respiratory activity of the decerebrate cat can become tolerant to the depressant action of morphine, after the drug has been in contact with nervous structures for a short period of time. Cross-tolerance between morphine and fentanyl was also evident. On the other hand, neither of the two drugs showed cross-tolerance with a barbiturate, thereby confirming the pharmacological specificity of the mechanism involved in the emergence of tolerance to these two types of drugs.

It must be pointed out that frequency and tidal volume showed a dissimilar course in the tolerance development. Regarding the respiratory frequency, the peak depression was already attained by the first dose; subsequent doses of morphine not only could not produce any lower values, but they induced a partial recovery. On the part of tidal volume, tolerance appeared after a longer latency, and no sign of recovery was evident by the end of the sixth hour. This divergence observed in the latency of the development of acute tolerance emphasizes the relative independence of the central mechanisms which generate the respiratory rhythmicity and amplitude. In studies of chronic tolerance performed in rats, KOKKA *et al.* (6) also showed that frequency could become tolerant to morphine earlier than the tidal volume, and that, as the morphine administration continued, the respiratory rate started to recover whereas the amplitude remained at a level lower than the control.

Since morphine depresses very specifically the central CO_2-detector system of the respiratory center, and the tidal volume is a direct function of the CO_2 level (2), it is possible that the derangement of central mechanisms may interfere more substantially with those involved in the generation of amplitude. In this regard, it is meaningful that the tidal volume responses to CO_2 were the function more consistently and lastingly depressed. But even in this situation the tolerance was evident, since doses of morphine given at a sequence which should bring about narcotic accumulation in the brain (11), did not produce increasing depression.

The pattern of acute tolerance described in this study is similar to that reported by COX *et al.* (1). In analgesic studies performed in awake rats, continuous infusion of morphine induced the development of

acute tolerance by the end of the second hour, and partial recovery of the analgesia at about the third hour. Other techniques have been used to establish a rapid state of tolerance (12). It follows that any theory proposed to account for the development of tolerance will have to consider the very short period of time needed to initiate the syndrome.

Acute dependence was clearly demonstrated by the effects induced by naloxone. Again, frequency and tidal volume were differently affected (Fig. 2). It means that the function which developed tolerance more easily, showed a greater degree of dependence, thus confirming the association between the two events. The new state reached after naloxone was not just a re-establishment of the control situation. Naloxone elicited the appearance of a state characterized by hyper-responsiveness of the respiratory center, as indicated by the shift to the left and the increase of the slope of the CO_2-ventilation relationship curves. Similar results have been described by MARTIN *et al.* (10) upon withdrawal of morphine in human addicts. Another important consideration is the fact that the minute volume responses to CO_2 remained depressed at a constant level during most of the experiment, and that the successive doses of morphine did not induce any phasic reduction of the response. Taken together, these effects lend support to the redundancy theory (9) proposed to explain some of the mechanisms involved in the development of acute and chronic tolerance and dependence.

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