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Effects of Morphine Upon the Pituitary-Adrenal System and Adrenal Catecholamines: A Comparative Study in Cats and Rats[1]

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GUAZA, C., A. TORRELLAS, J. BORRELL AND S. BORRELL. *Effects of morphine upon the pituitary-adrenal system and adrenal catecholamines: A comparative study in cats and rats.* PHARMAC. BIOCHEM. BEHAV. 11(1) 57–63, 1979.—The aim of the present study was to investigate the effects of acute and chronic administration of morphine upon the pituitary-adrenal activity and adrenal catecholamines in rats and cats, two animal species with very different behavioural patterns of response to the opiate. Acute administration of the drug induced in both animal species an activation of the pituitary-adrenal system. Chronic administration of morphine to cats and rats induced a depression in the pituitary-adrenal function. No significant changes in the adrenal levels of catecholamines were observed in rats treated chronically with the drug. However, in the cat, the effects of morphine on adrenomedullary function seemed to depend on the stage of morphine treatment. The behavioural patterns of response in both animal species during chronic administration of the opiate, as well as the effects of induced withdrawal with nalorphine (an antagonist of morphine), indicated that dependence on morphine had developed, not only in the rats, but also in the cats. Acute morphine administration had a sedative effect, while in the cats the opiate produced a species-specific manic response characterized by hyperexcitement and aggressive behavior.

Adrenal catecholamines Cat Corticosteroids Morphine Dependence on morphine Nalorphine Rat

IT IS well known that therapeutic doses of morphine produce analgesia and sedation in man and that several animal species, including rats, respond in the same fashion. In the rat, as in man, chronic treatment with morphine causes tolerance to the analgesic effect of the drug and physical dependence [14,16]. In other species, such as the cat, the prominent effects of morphine are usually regarded as excitatory and the development of tolerance and physical dependence on morphine in these animal species has been controversial [7, 13, 20, 25].

While extensive studies have been conducted on the effects of single or chronic morphine administration on the pituitary-adrenocortical system, as well as on the adrenal and brain catecholamines in the rat [1, 12, 19, 21, 27, 28, 30], data are scanty concerning the effects of morphine on animal species in which the drug has an excitatory effect [5, 10, 12, 19, 32].

The purpose of the present study is to examine the effects of acute and chronic morphine administration upon the pituitary-adrenal system and adrenal catecholamines in two different animal species-cats and rats. Aside from the behavioural differences in response to acute morphine administration in these species, important endocrine differences are also present. The rat's adrenal gland has a low percentage of norepinephrine [35], while the cat's has a relatively high percentage [4, 24, 35]. Cats and rats not only have different adrenal catecholamine levels, they also metabolize corticosteroids differently: in the rat the corticosteroid mainly secreted by the adrenal cortex is corticosterone, and in the cat it is cortisol [2, 3, 8, 31].

METHOD

Cats

Adult male and female cats, each weighing 2.5–3.0 kg, were used. The cats were kept in a quiet environment for at least 10 days prior to the experiments. The animals were killed by a blow on the head, immediately decapitated and the blood was collected from trunk vessels into heparinized tubes. Such an operation takes approximately one minute's

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time. Then the adrenals were quickly removed and weighed to the nearest mg. The blood was centrifuged and the plasma used for steroid determinations. Cats were treated intraperitoneally (IP) with either a single injection of morphine (morphine hydrochloride, Uquifa, S.A.E., Barcelona), or with daily injections of morphine for 7, 14 or 30 days: 10 mg/kg of the drug (as a dose of the free base) was injected in a volume of 0.5 ml: control cats received the same volume of saline. Some of the animals chronically treated with morphine for 13 days received a 10 mg dose of nalorphine (Uquifa, S.A.E., Barcelona) or saline 24 hr after their last injection of morphine. All cats were sacrificed 1 hr after the last injection and between 9:00 and 9:30 a.m., in order to eliminate the influence of diurnal variation.

Rats

Female Wistar rats, each weighing 200–300 g were used. The rats were housed four to a cage in a ventilated room at a temperature of about 25°C and with a 12 hr-day/night cycle. The animals were killed by decapitation with the aid of a guillotine and the blood was collected from trunk vessels into heparinized tubes: the adrenal glands were quickly removed and weighed. Rats were injected intraperitoneally with morphine (morphine hydrochloride, Uquifa, S.A.E., Barcelona) in a volume of 0.5 ml and the control animals received the same volume of saline. In the acute morphinization 50 mg/kg were injected and the animals were sacrificed 1 hr later. In the chronic treatment, rats were injected daily for 6 or 12 days. On the first day of treatment a single dose of 25 mg/kg of morphine was injected in the morning: on the following day rats were injected in the morning (9 a.m.) and in the afternoon (6 p.m.). On the second and third days, rats received two doses of 25 mg/kg of morphine: on the fourth and following days of treatment, two doses of 50 mg/kg were injected; and in the morning of the last day, according to the experimental group (6th or 12th day) a final dose of 50 mg/kg of the opiate was injected. Then the animals were sacrificed three hours after the last morning injection. To study the withdrawal from morphine, 10 mg/kg of nalorphine (Uquifa, S.A.E., Barcelona) was given immediately after the last injection of morphine. Then these animals were sacrificed 3 hr after the last injection of the antagonist. In order to eliminate the influence of diurnal variation, the animals were killed between 12 a.m. and 1 p.m.

Adrenal glands were homogenized in water for corticosteroid assays and in 0.01 N HCl for determinations of catecholamines. Adrenal and plasma corticosteroids, mainly represented by cortisol in the cat and by corticosterone in the rat, were measured as described by Matsumura *et al.* [17]. Adrenal norepinephrine (NE), epinephrine (E), and total catecholamines (CA) were determined by the method of Shore and Olin [26], as modified by Callingham and Cass [6]. Due to the fact that, in the rat adrenal glands, catecholamines are mainly represented by epinephrine [35], the results of the contents of these amines in the glands are expressed as epinephrine. However, the cat adrenal glands contain approximately equal parts of epinephrine and norepinephrine [4, 24, 35], and each bioamine was assayed individually. The total contents of catecholamines, as well as the E/NE ratio, were also evaluated. An Aminco Bowman spectrophotofluorometer was used. The data were evaluated statistically by the Student's t-test. A value of $p<0.05$ was considered statistically significant.

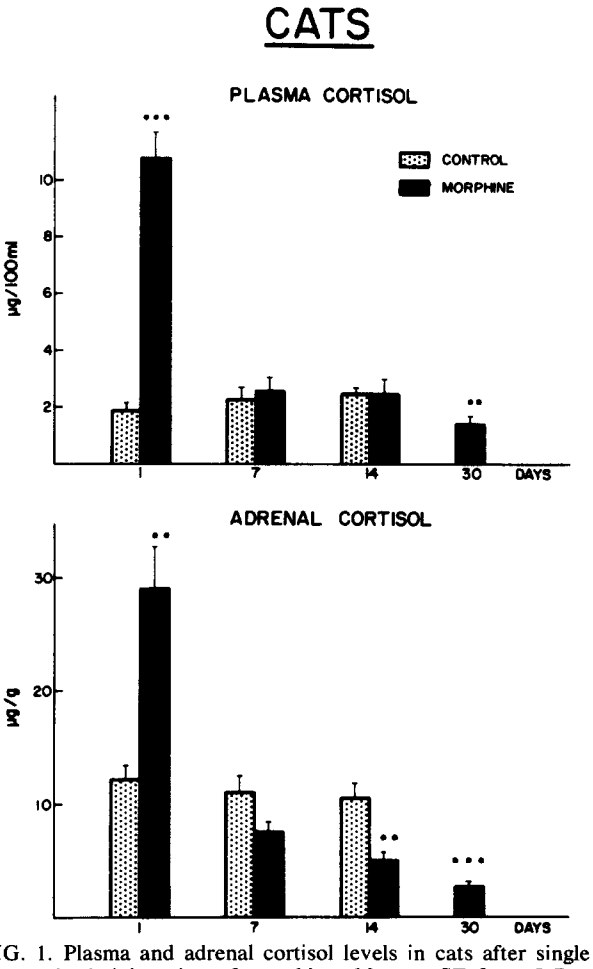

FIG. 1. Plasma and adrenal cortisol levels in cats after single or repeated administration of morphine. Mean ± SE from 5–7 cats. Animals were killed 1 hr after the last injection of the drug or saline. Significant differences from control cats: ***$p<0.001$; **$p<0.01$.

RESULTS

Cats

A single dose of 10 mg/kg of morphine induced in cats (Fig. 1) significant increases in the levels of adrenal and plasma cortisol ($p<0.01$ and $p<0.001$, respectively). Significant decreases in the adrenal level of norepinephrine ($p<0.01$) and epinephrine ($p<0.05$) were observed in these animals (Table 1). Also, a significant decrease ($p<0.01$) in the level of total catecholamines was observed when these animals were compared to the corresponding control cats. However, no significant variation sin the E/NE ratios were observed for these experimental groups (Table 1).

We observed significant differences in terms of adrenal epinephrine ($p<0.01$) and total catecholamine levels ($p<0.02$) between 1-day and 7-day control cats (Table 1). In our opinion these differences could be due to the handling and injection of the 1-day group of animals only once. Previous results in our laboratory indicated that cats injected once with saline and sacrificed 3 hr later had adrenal catecholamine levels within the same range as those obtained in 7-day control cats. Because of this, we considered it pertinent to perform 7-day and 14-day control groups. As no significant differences in adrenal catecholamine or ad-

TABLE 1

EFFECTS OF SINGLE AND REPEATED INJECTION OF MORPHINE ON CATECHOLAMINES LEVELS IN THE ADRENAL GLAND OF CATS

Treatment	NE (μg/g)	E (μg/g)	E/NE Ratio	Total CA (μg/g)
Control 1 day	1325 ± 112	1083 ± 137	0.82 ± 0.064	2407 ± 229
Morphine 1 day	712 ± 100†	648 ± 99§	0.95 ± 0.087	1382 ± 232†
Control 7 days	690 ± 69¶	773 ± 107	1.14 ± 0.112	1425 ± 232**
Morphine 7 days	1069 ± 74†	223 ± 54†	0.21 ± 0.050*	1296 ± 208
Control 14 days	771 ± 92¶	762 ± 58	1.03 ± 0.119	1533 ± 121**
Morphine 14 days	745 ± 87	613 ± 79	0.87 ± 0.105	1371 ± 194
Morphine 30 days	1134 ± 106§	1057 ± 110§	0.97 ± 0.131	2213 ± 173‡

Mean ± S.E. from 5–7 cats.

Significant differences from corresponding control cats: *$p<0.001$; †$p<0.01$; ‡$p<0.02$; §$p<0.05$.

Significant differences between different control groups: ¶$p<0.01$, **$p<0.02$ vs. control 1 day.

No significant differences were detected between 7 and 14-day control groups.

Animals were killed 1 hr after the last injection of the drug or saline.

TABLE 2

EFFECTS OF NALORPHINE-INDUCED WITHDRAWAL ON PLASMA AND ADRENAL CORTISOL AND CATECHOLAMINES IN THE CAT

	Cortisol		Adrenal catecholamines			
Treatment	Plasma (μg/100 ml)	Adrenal gland (μg/g)	NE (μg/g)	E (μg/g)	E/NE Ratio	Total CA (μg/g)
Saline 14 days	2.53 ± 0.21	10.64 ± 1.57	771 ± 92	762 ± 58	1.03 ± 0.119	1533 ± 121
Saline 13 days plus nalorphine	5.37 ± 1.01†	10.06 ± 1.19	855 ± 130	846 ± 96	1.03 ± 0.111	1701 ± 212
Morphine 14 days	2.53 ± 0.49	4.88 ± 0.71*	745 ± 87	613 ± 79	0.87 ± 0.106	1371 ± 194
Morphine 13 days plus saline	5.07 ± 0.94¶	10.32 ± 0.59‡	940 ± 131	581 ± 14	0.70 ± 0.122	1522 ± 221
Morphine 13 days plus nalorphine	14.55 ± 0.63‡	22.32 ± 2.80‡	1222 ± 158¶	390 ± 38¶	0.34 ± 0.050§	1612 ± 173

Mean ± S.E. from 5–7 cats.

Significant differences from saline 14 days: *$p<0.01$: †$p<0.05$.

Significant differences from morphinized 14 days: ‡$p<0.001$: §$p<0.01$: ¶$p<0.05$.

Animals were killed 1 hr after the last injection of the drugs or saline.

renal and plasma cortisol levels were observed between these two last groups of control cats (Table 1: Fig. 1), we did not perform the corresponding 30-day control cats.

The contents of cortisol in the adrenal glands after 7 days of daily morphine injection, although not significantly different, was lower than in the corresponding control animals (Fig. 1). After 14 consecutive days of daily injection of morphine, the adrenal cortisol decreased significantly ($p<0.01$) and this decrease was even more marked ($p<0.001$) after 30 days of treatment. Plasma cortisol decreased significantly ($p<0.001$) only after 30 days of daily morphine injection (Fig. 1). After 7 days of daily injection of morphine, a significant increase ($p<0.01$) in adrenal norepinephrine was found, together with a significant decrease ($p<0.01$) in epinephrine (Table 1). However, no significant differences in the total contents of adrenal catecholamines was observed, but the E/NE ratio in the adrenal glands was significantly ($p<0.001$) lower than the one observed in the corresponding control cats. After 2 weeks of daily morphine injection, the adrenal contents of norepinephrine, epinephrine and total catecholamines as well as the E/NE ratio were again within the range observed in the control animals (Table 1). The adrenal levels of both norepinephrine and epinephrine and, therefore, total catecholamines, in the cats subjected to morphine administration for one month (Table 1) were significantly higher than those detected in 1 or 14-day morphine treated cats as well as those levels in 7 or 14-day control groups of cats. However, no change in the E/NE ratio was observed in these animals.

In order to see whether tolerance to morphine in the cat had developed after 2 weeks of daily morphine injections (once the adrenal levels of epinephrine and norepinephrine

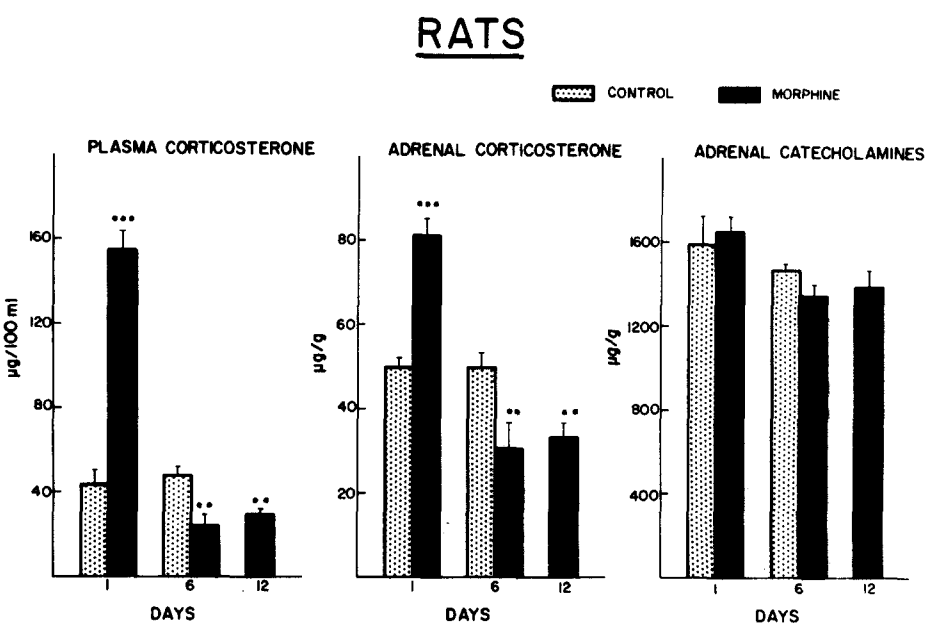

FIG. 2. Plasma and adrenal corticosterone and adrenal catecholamines in rats after single or repeated administration of morphine. Mean ± SE from 8–11 rats. Animals were killed 3 hr after the last injection of the drug or saline. Significant differences from control rats: ***$p<0.001$; **$p<0.01$.

had returned to control levels and a decrease in adrenal contents of cortisol was observed) nalorphine or saline was injected 24 hr after the 13th-day of daily morphine administration (Table 2). The animals were sacrificed 1 hr after the injection of the morphine antagonist or of saline. In comparison to the 14-day morphinized cats, the contents of adrenal and plasma cortisol increased significantly ($p<0.001$ and $p<0.05$, respectively) when saline was given and these increases were more marked ($p<0.001$) when nalorphine, instead of saline, was administered. Saline administration to morphinized cats did not cause significant changes in the levels of adrenal norepinephrine and epinephrine or in the E/NE ratio. But nalorphine caused a significant increase in norepinephrine ($p<0.05$), whereas a significant decrease in epinephrine ($p<0.05$) was observed when results were compared with the 14-day morphinized animals and, as a consequence, the E/NE ratio was significantly lower ($p<0.01$) than in control or in 14-day morphinized cats (Table 2). However, no significant difference was observed when the level of total catecholamines in the adrenal gland was evaluated.

Administration of nalorphine to cats injected for 13 days with saline (Table 2) did not cause any significant changes in the adrenal levels of norepinephrine or epinephrine. The small but significant increase ($p<0.05$) in the plasma cortisol level detected in this group of animals, when compared to 14-day saline injected cats, could reflect a partial agonist action of nalorphine. Nevertheless, the pituitary-adrenal activation induced by nalorphine seemed to be of little significance since the adrenal cortisol level was not significantly different from that observed in the corresponding control cats.

The behaviour of the animals was observed both before and after the injection of either morphine or nalorphine. When 10 mg/kg of morphine was administered to naive cats, the animals were extremely excited within 10–15 min following the injection and continuing for a minimum period of 8 hr. They were continuously restless, seemed frightened, avoided handling and produced loud prolonged cries. These symptoms and the length of duration began to wane gradually after 5–7 days of treatment with the opiate: some of the cats treated for 30 days with the drug still showed some hyperexcitability but this was much less intense than what was observed at the beginning of treatment. We interpreted this observation as a tolerance development to morphine in cats chronically treated with the opiate. Abrupt withdrawal induced by nalorphine caused an increase in the excitability of approximately the same magnitude as that observed in cats treated with the first injection of the drug. Nalorphine administration to the 13-day saline-treated cats did not induce any changes in the behaviour of the animals.

Rats

The administration of an initial dose of 50 mg/kg of morphine produced in the rat significant increases ($p<0.001$) in the adrenal and plasma corticosterone levels (Fig. 2). No significant changes were found in total adrenal catecholamines (Fig. 2). With chronic administration of the opiate (Fig. 2), significant decreases in plasma corticosterone were observed after 6 days ($p<0.01$) or 12 days ($p<0.01$). Adrenal levels of corticosterone were also lower than in control animals ($p<0.01$) in these two groups of experimental rats (Fig. 2). The levels of total adrenal catecholamines remained unchanged in these experimental animals (Fig. 2).

Nalorphine administered to rats that were daily injected with saline did not produce variations in the plasma corticosterone or adrenal catecholamine levels (Table 3). The administration of nalorphine to rats daily treated for 6 or 12 days with morphine induced significant increases ($p<0.001$) in the plasma corticosterone levels in comparison to the 6 or 12 day morphinized only rats. A significant reduction

TABLE 3

EFFECTS OF NALORPHINE-INDUCED WITHDRAWAL ON PLASMA CORTICOSTERONE AND ADRENAL CATECHOLAMINES IN THE RAT

Treatment	Corticosterone (μg/100 ml)	Catecholamines (μg/g)
Saline 6 days	48 ± 4	1455 ± 23
Saline 6 days plus nalorphine	56 ± 8	1252 ± 90
Morphine 6 days	24 ± 5*	1350 ± 58
Morphine 6 days plus nalorphine	65 ± 3†	1255 ± 109
Saline 12 days plus nalorphine	57 ± 3	1378 ± 80
Morphine 12 days	29 ± 3*	1390 ± 84
Morphine 12 days plus nalorphine	86 ± 6‡	719 ± 34‡

Mean ± S.E. from 8–11 rats.
Significant differences from saline 6 days: *$p<0.01$.
Significant diferences from morphinized 6 days: †$p<0.001$.
Significant differences from morphinized 12 days: ‡$p<0.001$.
Animals were killed 3 hrs after the last injection of the drug or saline.

($p<0.001$) in the adrenal level of catecholamines was observed when nalorphine was given to rats morphinized for 12 days only (Table 3).

After the administration of a single dose of morphine, the animals exhibited behavioural depression and catalepsy. When the treatment with the drug was prolonged, these effects changed to behavioural excitation and were stereotyped. During the withdrawal syndrome which followed the administration of nalorphine, we observed that the rats were extremely irritable and showed the typical withdrawal signs of tremor, pyloerection, diarrhea, wet-dog shakes and writhing. Nalorphine administration to the saline-treated rats did not induce any change in the behaviour of the animals.

DISCUSSION

The results reported here about the effects of acute morphine administration on the levels of adrenal and plasma corticosteroids in rats and cats are in agreement with the results of others who dealt with the activation of the pituitary-adrenocortical system, which was induced by the same drug, in non-tolerant animals [5, 15, 21, 22, 27, 29].

The release of catecholamines from the adrenal medulla after morphine treatment in different animal species has been attributed to the stimulation of certain receptors in the central nervous system and is mediated through the peripheral sympathetic nervous system [5,34]. In general, it is assumed that a single injection of morphine induces a depletion of adrenal amines, although most of the studies attempting to relate morphine effects to the adrenal catecholamines were concerned mostly with epinephrine. Our experiments indicate that in the cat a single dose of 10 mg/kg of morphine is able to induce a decrease in adrenal level of both norepinephrine and epinephrine, while in the rat a single dose of 50 mg/kg was not able to induce any signficiant change in the levels of total adrenal catecholamines. Although previous results in our laboratory (to be published) indicate that acute morphine administration in the rat induces an increase in the epinephrine turnover in the adrenal gland (without producing changes in their steady-state levels), our present results seem to indicate that the rat is more resistant to the catecholamine-releasing effect of morphine than the cat.

Although similar patterns in the effects of acute morphine administration on adrenal corticosteroid levels in cats and in rats were observed in our work, very different patterns in the behaviour of each animal species in response to the drug were observed. While in the cat an excitatory effect is observed, in the rat depression and catalepsy were induced by the drug. It has been reported that morphine produces in humans, monkeys, rats, hamsters, guinea pigs and rabbits a syndrome characterized by analgesia and sedation. However, in other species such as lions, cats, pigs, cows, goats and sheeps, the prominent effect of the opiate is usually regarded as excitatory [18]. On the other hand, from several investigations it has emerged that the proportion of adrenal epinephrine to norepinephrine differs widely among species [33]. Goodall [11] suggested that norepinephrine could act as a specific stimulating agent in carnivores and the high proportion of this hormone in feline animals seems to support this view. It should be taken into account that animals such as the rat, which respond to morphine administration with a syndrome characterized by analgesia and sedation generally show a higher E/NE adrenal ratio [33] than animals such as the cat, which respond to morphine with an excitatory syndrome. The question of whether or not the differences in the metabolism of adrenal catecholamines is related to the behavioural effects of morphine is unanswered.

The fact that chronic morphine administration induces a depression in the pituitary-adrenal function has been previously reported for animal species in which morphine has an analgesic effect [9, 15, 23, 29]. In our study, using the schedules of morphinization, tolerance to the stimulating effect of the opiate on ACTH secretion was present after a 6-day treatment in the rat and after a 1-week treatment in the cat. This fact indicates that a rapid tolerance to the stimulating effect of the opiate can be developed in both animal species irrespective of the different behavioural effects of the drug on each of these animal species.

Several authors [19,34] have reported increases in levels of adrenal catecholamines in chronically morphinized animals. In general, specially in rodents, due to the small percentage of norepinephrine present in their glands, the total of the catecholamines have usually been assayed instead of assaying epinephrine or norepinephrine separately. In our experiments on the rat, no changes in the levels of total catecholamines in the adrenal gland were observed during acute or chronic morphine administration. These results are in agreement with those reported by Gunne [12] who was unable to detect any change in the adrenal bioamines in either dogs or rats chronically treated with morphine. On the other hand, our results indicate that in the cat, when epinephrine and norepinephrine are evaluated separately, the effect of morphine on the adrenomedullary fuction depends on the stage of morphine treatment. A single injection of morphine caused a significant decrease in the adrenal contents of both epinephrine and norepinephrine, without change in the E/NE ratio. After administration of the same dose of the drug daily for 7 days, the normal E/NE ratio in the adrenal gland was profoundly altered in such a way that an increase in the contents of norepinephrine with a decrease in the contents of epinephrine was observed, although the total level of

catecholamines was within the range observed for control cats. After 14 days of daily morphine treatment, when some degree of physical dependence on the drug had been developed (see below), the contents of epinephrine and norepinephrine, as well as the E/NE ratio were again within normal levels. After 1 month of morphine treatment, an increase in the contents of both catecholamines was observed. If the disturbance in the contents of epinephrine and norepinephrine in the adrenal glands of cats which were subjected to daily morphine administration for 7 days is due to a reduction in epinephrine synthesis and/or to a selective secretion of bioamines from the adrenal medulla, and, if this effect could be related to the development of tolerance to morphine in the cat, then this should be the subject of further study.

The most effective way to induce the withdrawal syndrome in the early stages of development of dependence on morphine is by giving a narcotic antagonist such as nalorphine. After the injection of nalorphine into the morphinized cats or rats, a significant rise in the levels of plasma corticosteroids is observed. This fact may indicate that in both animal species some kind of dependence has developed after repeated administration of morphine, as has been suggested in other studies [10, 23, 29]. Catecholamine contents in the adrenal gland was significantly lower in the rats subject to morphine withdrawal after 12 days of daily injections of morphine. This finding agrees with results reported by others about the effects of nalorphine on morphine-tolerant dogs and rabbits [19]. A different response by the medullary catecholamines is observed in the cat: precipitated withdrawal with nalorphine in this animal species (as in the experimental group of animals subjected to morphine administration for 1 week) induced a disturbance in the E/NE adrenal ratio, although total contents of catecholamines is within the range obtained for the control animals and those only subjected to the chronic administration of morphine during 14 days. It remains to be established whether this alteration is mediated by a reduction in the synthesis of epinephrine or by a differentiated secretion of bioamines from the adrenal medulla.

The differences observed in the behaviour of the rats subjected to acute or chronic administration of morphine, or to nalorphine induced withdrawal, confirms the fact that in these animal species physical dependence on morphine develops. Although the behaviour pattern of cats in response to acute morphine administration is very different from that observed in rats, our results are in agreement with the point of view [13,20] that physical dependence on morphine also occurs in cats treated chronically with the opiate, and that a withdrawal-like syndrome can be precipitated in this animal species when the narcotic antagonist, nalorphine, is administered.

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