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REVERSAL OF THE ACTION OF MORPHINE ON THE SECRETORY ACTIVITY OF THE ADRENAL CORTEX

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Abstract—The effect of morphine on the secretory activity of the adrenal cortex was studied in mice, rats and rabbits. In mice and rats a single dose of morphine increased the level of plasma corticosterone In rabbits this rise was observed only in summer and in winter morphine reduced the level of plasma corticosterone. The repeated administration of morphine led to the development of tolerance towards its stimulating action. In addition, the basal level of corticosterone in plasma was significantly higher in chronically treated animals than in controls. After a sufficient period of chronic treatment with morphine, animals tolerant to the drug responded to a further dose with a decrease of plasma corticosterone. This reversal of action was particularly striking in rabbits. After withdrawal, the disorders that occurred during the period of chronic morphine administration persisted for a long time. The results obtained from the histochemical and biochemical study of the adrenal tissue are generally in good agreement with the changes observed in the plasma.

THE ADMINISTRATION of morphine to the rabbit, the rat and the mouse leads to metabolic disturbances correlated with changes in various endocrine secretions.[1] Many experiments have been done to solve this problem, but they were usually restricted to the role of the adrenal medulla in the post morphinic hyperglycemia or to that of the adrenal-hypophysis axis in the mechanism of analgesia. Many authors have thus described an ACTH release following an injection of morphine, leading to a stimulation of the adrenal cortex.

Moreover, the administration of this drug is known to lead to the development of tolerance and physical addiction phenomena. We have determined the evolution of the effects of morphine on corticosteroid secretion when regular injections of the narcotic were given for various periods of time, followed by a period of withdrawal.

MATERIALS AND METHODS

Animals. Three species of animals were used: male common grey rabbits, average weight 2·5 kg; male Wistar rats weighing 250–300 g, and male Swiss mice aged 6–8 weeks. Blood samples were taken from the marginal vein of the ear in rabbits, or after sectioning the jugular vein in the rat and the mouse. Morphine chlorhydrate was administered intramuscularly. Drug doses will be specified later.

Assays. The plasma corticosterone level in all animals was determined according to a spectrofluorimetric method of Zenker and Bernstein.[2] In rabbits and rats the adrenals were also studied histochemically. Glands were fixed in formaldehyde-calcium, cut into 10 μm thick slices by the congelation technique, then stained with Sudan black B or viewed under polarized light. The histochemical changes in rats were then specified by determining tissue corticosterone concentrations. In addition,

the lipid fractions of the cortex were separated by thin-layer chromatography on silica gel, followed by densitometry after sulfuric acid (10 per cent) charring. Using this method, an estimate with an accuracy of ± 10–15 per cent was made which was considered sufficient for preliminary determinations.

RESULTS

Plasma corticosterone

Effects of a single dose of morphine (10 mg/kg) in the rabbit. The effect of morphine on the plasma corticosterone level was variable and appeared to be closely linked to an annual cycle. Observations were made on 64 rabbits; blood samples were taken at 09·00 hr from 15 hr-fasted animals. Results are shown in Fig. 1. The level of corticosterone in normal animals varied little during the year but very significant differences in the response to morphine were observed depending on the season. In July, there was an elevation of the level of plasma corticosterone in all animals 1 hr following the injection of 10 mg/kg. Plasma corticosterone levels sometimes trebled but the average increase was about 83 per cent ($P < 0{\cdot}001$). In January, the opposite effect was observed, since the same dose of morphine induced a 30 per cent decrease in plasma corticosterone ($P < 0{\cdot}05$). Finally, in the intermediate periods of March and October, animals exhibited marked individual variations in the response to morphine injection; the mean values for plasma corticosterone following morphine injection were not significantly different from the control values.

Chronic morphinized and abstinent rabbits. The experiment was conducted in summer. Daily increasing doses of morphine chlorhydrate ranging from 10 to 20 mg/kg,

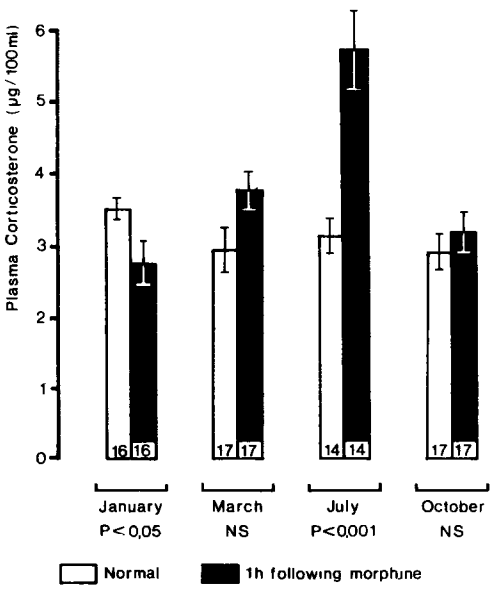

FIG. 1. Influence of the season on the effect of the first injection of morphine (10 mg/kg, i.m.) on the plasma corticosterone level in the rabbit. The results are plotted as mean ± S.E., the numbers in the bars indicate the number of animals. Significance was determined by the Student's *t*-test.

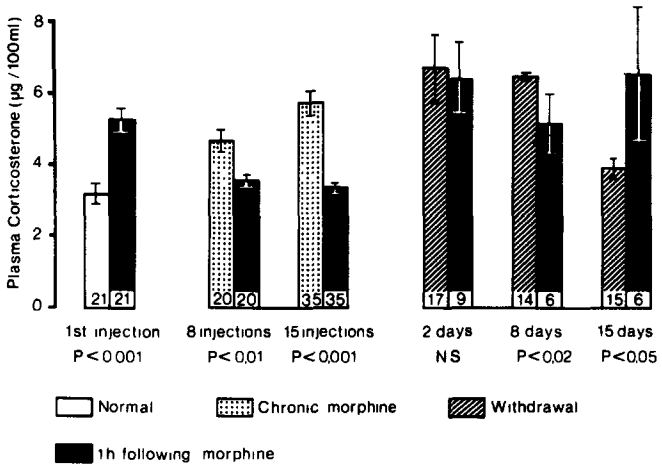

FIG. 2. Evolution of the changes in the plasma corticosterone levels in the rabbit during chronic morphine treatment (10–20 mg/kg/day for 15 days) and after withdrawal. The results are plotted as mean ± S.E., the numbers in the bars indicate the number of animals. Significance was determined by the Student's *t*-test.

during 15 days, were injected into rabbits. Samples of blood were taken 24 hr after the last injection and 1 hr following a further dose of morphine. Results are shown in Fig. 2.

Two striking results were observed; first, an increase in the corticosterone level in the blood sample taken immediately before the new injection. After the 15th injection, the level of corticosterone was nearly twice as high as the initial level in normal animals. The elevated value of corticosterone persisted at least 8 days after the cessation of regular treatment with morphine. In chronic morphinized animals a clear reversal of the initial effect of the drug was then observed. Each new dose no longer increased the level of corticosterone, but resulted in a significant decrease. The rate of the reversal of action varied according to the individual animal. Further studies indicate that this reversal of action takes place during the fifth day of treatment. However, after 8 injections the phenomenon became generalized and persisted if the chronic intoxication was continued, which, in our experiments, was limited to 15 days.

Two days following withdrawal morphine appeared to be devoid of action due to individual animal variations, although on the 8th day a clear reversal of action was noticed. Finally, 15 days after withdrawal, a further injection of morphine induced again hypercorticosteronemia as in control animals.

Effects of morphine in the rat and the mouse. In these two species no seasonal influences were noted. In the rat, the level of corticosterone in plasma was nearly 10 times as high as in the rabbit (Table 1). A single dose of morphine (20 mg/kg) induced a significant increase, the plasma corticosterone level being 50 per cent higher 1 hr following injection. After chronic administration of progressively increasing doses, the basal level of plasma corticosterone was 40 per cent higher on an average than that of normal rats. Finally, tolerance to the initial effect and reversal of action occurred.

TABLE 1. EFFECT OF MORPHINE ON THE PLASMA CORTICOSTERONE LEVEL OF NORMAL, CHRONIC MORPHINIZED AND ABSTINENT RATS

	Plasma corticosterone (μg/100 ml)*			
	Before morphine		1 hr following morphine	
1st Injection	35·8 ± 1·9 (22)	P < 0·01	58·5 ± 4·6 (20)	(20 mg/kg)
8th Injection	51·5 ± 4·4 (11)	NS	43·5 ± 4·2 (14)	(20 mg/kg)
21st Injection	51·6 ± 2·7 (11)	P < 0·05	35·9 ± 5·5 (8)	(50 mg/kg)
8 Days after withdrawal	39·4 ± 6·5 (8)	NS	43·4 ± 5·9 (8)	(20 mg/kg)

* Values are means ± S.E. The number of animals is shown in parentheses. Significance was determined by the student's *t*-test. NS = not significant.

The use of rats with intra-aortic cannulas allowed us to compare the levels before and after morphine in the same animal, and monitor the individual variations in response. These results support those obtained by using groups of animals.

After withdrawal the level of plasma corticosterone returned to normal; but after 8 days the tolerance persisted, since a further injection of morphine had no immediate and significant effect on the level of corticosterone.

In the mouse, the determinations made on a group of 89 animals showed that morphine also stimulates significantly the secretion of corticoids. The normal level of corticosterone, which is 26·2 ± 2·5 μg/100 ml of plasma, rose to 63·5 ± 3·7 ($P < 0·001$) one hour following an initial dose of morphine (100 mg/kg, i.m.). Chronic morphine treatment, as in the rat, induced a plasma corticosterone level higher than that observed in normal animals ($P < 0·001$). Indeed, after 10 days of daily injections, doses ranging from 25 to 100 mg/kg, the plasma level of corticosterone was 44·4 ± 3·4 μg/100 ml in the blood sample taken 24 hr after the last injection, instead of 26·2 in the normal animal. This mean value rose to 61·7 ± 4·7 μg 1 hr after a further dose of morphine (100 mg/kg), showing that the stimulating action persisted, but to a lesser extent. No reversal of action was seen in mice.

Study of the cortico-adrenal tissue

Histochemical examination. The interesting results described above called for a detailed study of the effects of morphine at the level of the adrenal cortex tissue. A study of birefringent lipids gives some information about the activity of the adrenal cortex; the stimulation of secretion is known to lead in most cases to a depletion of these lipids and an accumulation of lipids may indicate a decrease in the secretory activity of the tissue.

A morphological study was carried out in the rat. Samples were taken from groups of animals at 10, 30, 60 min and then 2, 6, 12 and 24 hr after the administration of morphine (10 mg/kg). There were six rats in each group.

Approximately 10–30 min after the administration of morphine, the morphological study showed a shrinking of the reticulated zone and a migration of lipid vacuoles from the interior to the periphery of the fasciculated zone. The changes in the amount of birefringent lipids were typical; a brief but important accumulation was seen, followed by a distinct depletion about 1 hr later, and eventually a return to the initial appearance 6 hr after the first injection. During 1 week of chronic morphine treatment, the drug maintained its initial action on the adrenal cortex, but later a progressively greater accumulation of birefringent lipids was noticed. Consequently, it became difficult to detect an immediate effect of the drug.

Following withdrawal, the accumulation of birefringent lipids was evident beyond the 15th day after the regular treatment had been stopped.

In the rabbit a less extensive study was carried out, but the results obtained confirm those described in the rat.

Corticosterone and lipids of the adrenal cortex in the rat. Our aim was to investigate the lipid fraction in which the histochemical changes occur. We therefore determined the corticosterone level using the spectrofluorimetric technique and also the lipid fractions by thin-layer chromatography followed by densitometry. The analyses were made on adrenals from normal rats before and one hour following an injection of morphine (20 mg/kg i.m.), in rats chronically morphinized for 2 weeks by injecting them with increasing doses (20–60 mg/kg), and in 1 week-abstinent rats.

TABLE 2. EFFECTS OF MORPHINE ON THE CORTICOSTERONE AND ON THE FREE OR ESTERIFIED CHOLESTEROL LEVELS OF RAT ADRENAL CORTEX

	S/C (× 10^6)	Corticosterone (μg/100 mg wet wt)	Esterified cholesterol (mg/100 mg wet wt)	Free cholesterol (mg/100 mg wet wt)
Normal rats				
Before morphine	108 (8)	4·4 ± 0·7* (8)	2·53 ± 0·13 (15)	0·72 ± 0·03 (15)
1 hr Following morphine (20 mg/kg)	120 (8)	4·8 ± 0·5 (8)	2·04 ± 0·37 (8)	0·67 ± 0·05 (8)
Chronic morphinized rats (20–60 mg/kg/day/15 days)				
24 hr After the last injection	196 (8)	5·1 ± 0·5 (8)	1·84 ± 0·12 (8)	0·72 ± 0·03 (17)
1 hr Following a further dose	182 (8)	5·6 ± 0·5 (8)	2·93 ± 0·33 (14)	0·65 ± 0·03 (14)
Abstinent rats				
8 days after the last injection	156 (5)	3·9 ± 1·2 (5)	2·09 ± 0·25 (7)	0·64 ± 0·04 (7)
1 hr Following a further dose (20 mg/kg)	139 (6)	6·3 ± 0·7 (6)	2·50 ± 0·36 (6)	0·62 ± 0·06 (6)

* Values are means ± S.E. The number of animals is shown in parentheses.
S/C = adrenal wt/body wt.

No significant changes in the concentrations of phospholipids and triglycerides were detected in any of the rats.

The effects of morphine on the cholesterol and the corticosterone levels in adrenal cortex are shown in Table 2. Hypertrophy of the adrenals was noted in chronically morphinized rats, a phenomenon that was previously described[3] and which also persists after withdrawal. There was no change in the concentration of free cholesterol. The concentration of cholesterol esters changed significantly, while that of corticosterone changed only slightly during chronic treatment with morphine.

DISCUSSION

The effect of a single dose of morphine has been proved by the determination of plasma corticosterone concentration, by the histochemical study of the adrenal cortex and, in the case of the rat, by the determination of the lipid and corticosterone content of the adrenal cortex. The initial administration of morphine increased the secretion of corticosterone in all three species; in the rabbit, this effect was distinctly linked to a seasonal cycle. In the rat and the mouse the stimulating effect of morphine on the secretion of corticosterone occurred through the whole year and this is consistent with the previously published data. However few investigators have examined the changes in plasma corticosterone levels following morphine administration. The main effect studied has been the ascorbic acid depletion in the adrenal glands. Determinations of plasma steroids have been made mainly in man, and results are variable. Eisenman *et al.*[4] observed an inhibition of corticosteroid secretion following morphine administration, whereas Fraser *et al.*[5] did not detect any response in the adrenals. Our results support those of Suzuki *et al.*[6] described for the dog, since the secretion rate of steroids clearly increases in this animal during the 120 min following the injection of morphine. Similar effects were noticed in the monkey.[7] In addition recent work[8] has shown an increase in the level of corticosterone after administration of morphine in the rat, which appears to be followed by an increased excretion of urinary steroids.[9] We noticed a transitory lipid accumulation followed by a depletion of birefringent lipids in the adrenal cortex, and this agrees well with the high corticosterone levels that were found. Morphine would thus affect the adrenal cortex by initially inducing a synthesis, and then an increased release of corticosteroids, probably mediated by ACTH. Further experiments are needed to determine the precise nature of the lipids involved.

Our results, and those obtained by other investigators, suggest that morphine almost always induces a secretion of corticosterone when first injected. However, it should be noted that under certain conditions morphine can induce a significant lowering of the level of plasma corticosterone. We found that seasonal influences and some unknown form of adaptation to chronic morphine treatment reversed the action of morphine. The narcotic clearly leads to a hypercorticosteronemia in summer in the rabbit, whereas in winter there is a complete reversal of this action. During spring and autumn, the effects vary with the animals. These changes might be linked to the degree of adrenal tissue sensitivity to ACTH. In accordance with this concept, Boright *et al*[10] have noted a variable sensitivity of adipose tissue to ACTH action. These authors noticed a very weak lipolytic activity of ACTH in winter, an activity which

becomes distinctly higher in summer and which appears to be variable in autumn and spring. Yet if a relationship exists between the variable activity of the adrenal cortex and the modified sensitivity of the tissue to ACTH, there is still no explanation for the mechanism of the reversal of action of morphine in winter.

Reversal of morphine action was also found during chronic morphine treatment, particularly in the rabbit. The depression of plasma levels of corticosterone was as much as 50 per cent 1 hr following the 15th daily injection. This phenomenon of reversal of action is as yet unexplained. Histochemical studies do not show great changes in the appearance of adrenal cortex slices. We noticed an unexpectedly small but significant accumulation of cholesterol esters ($P < 0{\cdot}001$) in the adrenal cortex during chronic morphine treatment. One week after withdrawal, this accumulation of esters persisted ($P < 0{\cdot}02$). The meaning of these changes in adrenal cortex lipids is still unclear.

The existence of these reversals of action occurring during chronic morphine treatment seems to be general, since Schmid[11] described a similar phenomenon in the case of blood sugar in chronic morphinized dogs, and we noted the effect on various plasma constituents in the rabbit.[12]

In the rat the reversal of action does not occur as often but tolerance rapidly develops in rats towards the immediate effect of the drug. The phenomenon may be linked to the inhibition of ACTH secretion described in the rat[13]; but it might also be due to a lowering of the adrenal cortex sensitivity to ACTH action in chronic morphinized animals. In support of this hypothesis, it should be noted that the *in vitro* synthetic ability of adrenal cortex slices taken from morphinized rats is reduced, and that the tissue shows little response when ACTH is added.[8]

Plasma corticosterone concentrations in chronically morphinized rats, rabbits and mice are significantly (2–3 times) higher 24 hr following the last injection, than in normal animals. Histochemical investigations show an accumulation of birefringent lipids in the adrenal cortex, which indicates either an inhibition of release or an increased synthesis of steroids in the tissue. The high level of corticosterone in plasma might also be linked to a lowering of the catabolism of steroids, which would lead to a reduced excretion of urinary steroids in chronic morphinized men[14] and rats.[9]

Our experiments show that the changes due to chronic morphine treatment may persist for a long time after withdrawal. The lipid accumulation in the adrenal cortex diminishes very gradually, since even 15 days after cessation of morphine administration, the activity of the adrenals had not returned to normal in the rat. The depletion of the adrenal lipids noticed at that time is consistent with an increased urinary excretion of seroids which has been observed during the first few days of withdrawal from chronic morphine treatment in human subjects and in rats.

To conclude, the effects of morphine on the secretion of corticosteroids seem to be variable and complex, leading to profound changes in the activity of the adrenal cortex. It is remarkable that these disturbances can last so long after withdrawal. The existence of the reversal of action of morphine, which initially induces a stimulation of the secretion of corticosterone, and subsequently a lowering of the level of corticosterone secretion, appears to be an important phenomenon. There is a profound modification of the physiological equilibrium which occurs in chronic morphinized animals and which could logically be correlated with the manifestations of the withdrawal syndrome.

REFERENCES

1. R. SABLE-AMPLIS, R. AGID et D. ABADIE, *J. Physiol., Paris* **63,** 282 (1971).
2. N. ZENKER and E. BERNSTEIN, *J. biol. Chem.* **231,** 695 (1958).
3. E. M. MCKAY, and L. L. MCKAY, *Arch. Int. Pharmacodyn.* **52,** 363 (1926).
4. A. J. EISENMAN, H. F. FRASER, J. SLOAN, and H. ISBELL, *J. Pharmac. exp. Ther.* **124,** 305 (1958).
5. H. F. FRASER, G. D. VANHORN, and H. ISBELL, *Am. J. Med. Sci* **231,** 1 (1956).
6. T. SUZUKI, K. YAMASHITA, S. ZINNOUCHI and T. MITAMURA, *Nature, Lond.* **183,** 825 (1959).
7. J. N. MASON, C. T. HARWOOD, and N. R. ROSENTHAL, *Am. J. Physiol.* **190,** 429 (1957).
8. M. HIRAI, K. N. LIN, and T. NAKAO, *Endocrinol. Japan* **17,** 65 (1970).
9. E. PAROLI, and P. MELCHIORRI, *Biochem. Pharmac.* **6,** 18 (1961).
10. H. A. BORIGHT, F. L. ENGEL, H. E. LEBOVITZ, J. L. KOSTYO, and J. E. WHITE, *Biochem. J.* **83,** 95 (1962).
11. F. SCHMID, *C. R. Soc. Biol., Paris* **147,** 129 (1953).
12. R. SABLE-AMPLIS, R. AGID et D. ABADIE, *J. Physiol., Paris* **59,** 292 (1967).
13. F. N. BRIGGS and P. L. MUNSON, *Endocrinology* **57,** 205 (1955).
14. A. J. EISENMAN, H. J. FRASER and J. N. BROOKS, *J. Pharmac. exp. Ther.* **132,** 226 (1961).