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Morphine analgesia, tolerance and physical dependence in the adrenalectomized rat

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Summary

1. Adrenalectomy reduced the median antinociceptive dose (AD50) of morphine in male Sprague–Dawley rats. The antinociceptive effect was assessed by the tail-flick method of D'Amour & Smith (1941).

2. Tolerance to the antinociceptive effect of morphine developed in adrenalectomized and sham-operated rats after chronic exposure to morphine. Development of tolerance did not significantly alter the increased sensitivity of adrenalectomized rats to the antinociceptive effect of morphine.

3. Adrenal weights were not increased in rats rendered physically dependent on morphine by subcutaneous implantation of a morphine pellet. Withdrawal, induced by intraperitoneal injection of naloxone hydrochloride, 4 mg/kg, or by removal of the implanted pellet, resulted in a rapid increase in adrenal weight.

4. In morphine-dependent animals, the incidence of abstinence signs and body weight loss during precipitated withdrawal did not appear to be significantly influenced by adrenalectomy or by corticosterone-pretreatment.

Introduction

The acute and chronic effects of morphine on the pituitary-adrenal axis have been extensively studied (Sloan, 1971 ; George, 1971). In earlier investigations Lewis (1923) and MacKay & MacKay (1929) noted that bilateral adrenalectomy increased the susceptibility of rats to the lethal effects of morphine. Hypertrophied adrenal glands have been observed in rats chronically treated with morphine (MacKay, 1931 ; Sung, Way & Scott, 1953). Akera & Brody (1968), however, found no increase in adrenal weights of morphine-dependent rats when the morphine dosage was gradually increased on a 8 h interval, 49 day injection schedule. Chronic morphine administration is accompanied by a decreased urinary excretion of corticosteroids in man (Eisenman, Fraser & Brooks, 1961) and in rats (Paroli & Melchiorri, 1961). Withdrawal raises urinary steroid levels in both species (Sloan, 1971).

The possibility that adaptive processes to morphine are mediated by the pituitary-adrenal axis was considered by MacKay (1931) and by Sung *et al.* (1953). Selye (1937), however, found that adrenalectomy did not abolish acquired tolerance to the lethal effects of morphine. Way, Sung & Fujimoto (1954) and Gebhart & Mitchell (1972) have also shown that the development of tolerance to the antinociceptive effects of morphine in rats is not inhibited by adrenalectomy. Tanabe & Cafruny (1958) reported that morphine withdrawal in 6 hypophysectomized rats was similar in intensity and duration to that observed in unoperated controls. In order to clarify and confirm some of these findings, the antinociceptive effect of

morphine, tolerance and physical dependence in the adrenalectomized rat was examined in the present investigation. The object of the experiments was to determine whether some of the pharmacodynamic actions of morphine, namely, analgesia, tolerance, and physical dependence, are mediated by the pituitary-adrenal axis.

Methods

Male Sprague–Dawley rats (Simonsen Laboratories, Gilroy, California), weighing 200–250 g, were used throughout these experiments. Animals were housed two to a cage under a 12 h light–dark cycle with food and water available *ad libitum*. Adrenalectomy or sham operations were performed by bilateral dorsal incisions under ether anaesthesia. Operated animals were given 0·9% w/v NaCl solution (saline) as drinking water for 7 days before experimentation. At the end of the experiment the surgical removal of adrenal tissue was confirmed by gross autopsy.

The antinociceptive effect of subcutaneously administered morphine was assessed by the method of D'Amour & Smith (1941). Thirty minutes after injection, heat generated by a 30 W spot-lamp was focused on the tail, 2–5 cm distal from the tip. In 8 sham-operated rats injected with saline, the tail-flick response was observed within $4{\cdot}2 \pm 0{\cdot}2$ (S.E.M.) s (range 2·5 to 7·2 s), after the lamp was switched on. An animal was considered analgesic if no tail-flick response was observed within 10 seconds. The median dose (AD50), 95% confidence limits and slope of the dose-response curve were calculated (Litchfield & Wilcoxon, 1949).

Tolerance to and physical dependence on, morphine were induced by subcutaneous implantation, under light ether anaesthesia, of a pellet containing 75 mg of morphine base (Gibson & Tingstad, 1970). In the tolerance experiments, rats were implanted for 48 h and the pellet removed under ether anaesthesia. The antinociceptive effect of morphine was determined at 24 and 48 h after pellet removal. Because of the narrow range of the dose-response curve, repeated injections of morphine sulphate were necessary to estimate the AD50 value for morphine. Animals were again randomly selected for the second test so that possible drug-test interactions were minimized (Kayan, Woods & Mitchell, 1969). At least 8 animals were tested at each of the 4 dose levels of morphine that were studied.

Withdrawal was precipitated by intraperitoneal injection of the antagonist, naloxone hydrochloride, 70–72 h after pellet implantation. The degree of physical dependence was assessed by methods that have been described elsewhere in detail (Wei, 1973; Wei, Loh & Way, 1973). Briefly, rats were weighed and placed in 3·8 litre jars. After an adjustment period of 10–15 min, naloxone was administered and behaviour observed for 10 minutes. The presence or absence of diarrhoea was recorded 30–45 min after naloxone. Behaviour was ranked according to Table 1. Animals were reweighed 3 h after injection of naloxone to calculate body weight loss during withdrawal. The rationale and validity of this assessment procedure is based on the relative magnitude of the median effective dose of naloxone which

TABLE 1. *A ranking system for the assessment of precipitated abstinence in rats*

Abstinence behaviour	Abstinence rank
No change in behaviour	0
Abnormal posturing, ear blanching, diarrhoea (any 2 out of these 3 abstinence signs)	1
Teeth chattering or swallowing movements or salivation	2
Two or more escape attempts or 3 or more wet shakes	3

precipitates these abstinence signs in the morphine-dependent rat (Wei, 1973; Wei, *et al.*, 1973).

In one experiment, precipitation of withdrawal was preceded by pretreatment of animals with corticosterone suspended in corn oil, 15 mg/kg s.c. at 08 h 00 min–09 h 00 min and 35 mg/kg s.c. at 16 h 00 min–18 h 00 min, for 3 days after implantation of the morphine pellet. Controls received corn oil only. The dose of corticosterone employed was similar to that used by several investigators to raise plasma corticosterone levels in the rat (Radzialowski & Bousquet, 1968; Wei & Wilson, 1971).

At the end of the experiment, the animals were killed by cervical dislocation. Student's t test was used to test differences in adrenal and body weights and the Mann–Whitney U test to assess differences in abstinence rank (Siegel, 1956). A probability of less than 0·05 was considered statistically significant.

Drugs

Morphine sulphate, naloxone hydrochloride (Endo Laboratories) were dissolved in saline; the doses refer to the salts. Corticosterone was suspended in corn oil. All drugs were administered in volumes of 0·1 ml/100 g body weight.

Results

Adrenalectomized rats were more sensitive to the antinociceptive action of morphine than control rats (Table 2). The AD50 of morphine in sham-operated rats was approximately twice the value found in adrenalectomized rats. The morphine effect was more variable in adrenalectomized rats than in controls; the increase in variability was perhaps associated with the hypoactivity of the adrenalectomized rats.

Chronic exposure to morphine resulted in the development of tolerance in adrenalectomized and sham-operated rats (Table 2). Eight of the 40 adrenalectomized rats treated chronically with morphine died during the experiment; the increased susceptibility of adrenalectomized rats to the lethal effects of morphine has been noted by MacKay & MacKay (1929). The adrenalectomized animals were more sensitive to the antinociceptive effect of morphine than the operated controls, although tolerance developed in both groups (Table 2).

Subcutaneous implantation of a morphine pellet rapidly induced physical dependence as evidenced by the withdrawal syndrome precipitated by naloxone

TABLE 2. *Antinociceptive effect of morphine in adrenalectomized and sham-operated rats*

Treatment	Non-tolerant rats		Tolerant rats	
	AD50 (mg/kg)	Slope	AD50 (mg/kg)	Slope
Sham-operated	3·9 (3·3–4·6)	1·4 (1·2–1·6)	9·6 (8·7–10·6)*	1·3 (1·2–1·5)
Adrenalectomized	1·6 (1·0–2·5)†	2·3 (1·3–4·1)	6·7 (5·2–8·7)*†	1·7 (1·2–2·4)

The median dose (AD50) and slope of the dose-response curve for morphine was calculated according to the method of Litchfield & Wilcoxon (1949). Eight to twelve rats were tested at each of the 4 dose levels of morphine. Numbers in parentheses are the 95% confidence limits. Tolerance to morphine was induced by subcutaneous implantation, for 48 h, of a pellet containing 75 mg of morphine base. Eight out of the 40 adrenalectomized rats treated chronically with morphine died during the experiment. *$P<0·05$, AD50 values for tolerant versus non-tolerant group. †$P<0·05$, AD50 values for adrenalectomized versus sham-operated group.

TABLE 3. *Intensity of abstinence in tolerant rats after naloxone*

Treatment	No. of animals	Abstinence rank 0	1	2	3	Mean abstinence rank
			(%)			
Placebo	23	91	0	9	0	0·2
Morphine	23	4	0	9	87	2·8*

Physical dependence on morphine was produced by implanting subcutaneously, for 72 h, a pellet containing 75 mg of morphine base. Controls were implanted with pellets containing only the excipient. Behaviour indicating abstinence was observed for 10 min after injection of naloxone hydrochloride (4 mg/kg, i.p.). *$P<0.05$, Mann-Whitney U test.

(Table 3). Development of dependence was not accompanied by any increase in adrenal weight; on the other hand, the stress of abstinence, precipitated by naloxone or by removal of the morphine pellet, rapidly increased adrenal weight in the morphine-dependent rat (Fig. 1).

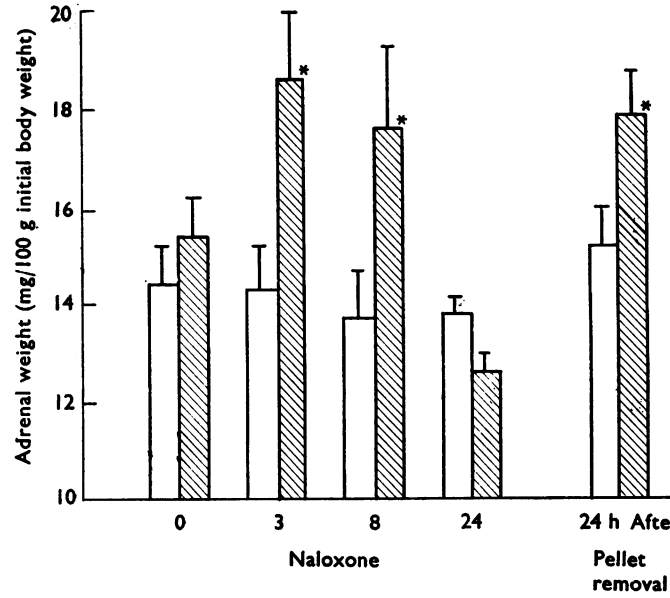

FIG. 1. Adrenal weights (both glands) after precipitated withdrawal in morphine-dependent rats. Physical dependence on morphine induced by subcutaneous implantation for 72 h of a pellet containing 75 mg of morphine base (open columns—placebo pellet; cross-hatched columns—morphine pellet). The values at 0 and 3 h after naloxone hydrochloride (4 mg/kg i.p.) and at 24 h after pellet removal are the mean values from 10 animals; the values at 8 h were obtained on 6 rats and those at 24 h on 7 rats. Vertical bars indicate the S.E.M. *$P<0.05$ for implanted versus control rats.

The adrenal weights are recorded as mg/100 g of the body weight at the time of naloxone injection or pellet removal because the loss of body weight (Fig. 2) is a characteristic feature of the abstinence syndrome in the rat (Goode, 1971). Implantation of the morphine pellet did not alter body weight (placebo 231 ± 3 g; morphine implanted 227 ± 4 g).

The degree of physical dependence in adrenalectomized or corticosterone treated rats is compared to their respective controls in Table 4. No significant difference, attributable to adrenalectomy or corticosterone treatment, could be found when behavioural parameters and body weight loss were used as quantitative indices of physical dependence.

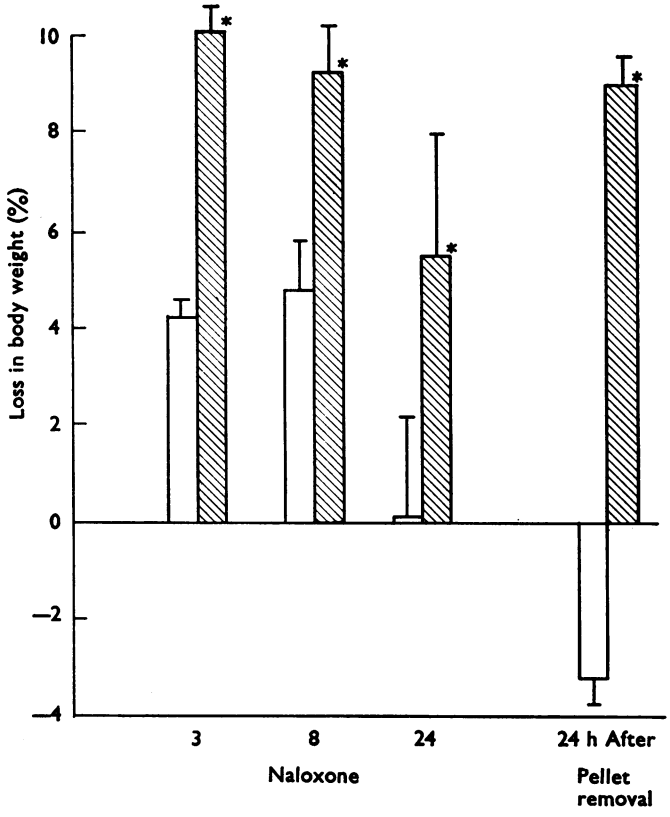

FIG. 2. Body weight loss after precipitated withdrawal in morphine-dependent rats. Experimental procedures are described in the legend to Fig. 1. Open columns—placebo pellet; cross-hatched columns—morphine pellet. *$P<0.05$ for implanted versus control rats.

TABLE 4. *Intensity of abstinence after naloxone in adrenalectomized or corticosterone-treated rats*

Treatment	No. of animals	Abstinence rank 0	1	2	3	Mean abstinence rank	Loss in body weight (%)
			(%)				
Sham-operated	14	36	14	14	36	1·5	5·1±0·5
Adrenalectomized	12	33	17	8	42	1·6	4·0±0·7
Corn oil	12	42	0	8	50	1·7	5·4±0·2
Corticosterone	12	42	0	0	58	1·7	4·7±0·5

Abstinence was precipitated 72 h after subcutaneous implantation of a pellet containing 75 mg of morphine base. Corticosterone was administered subcutaneously, 15 mg/kg at 08 h 00 min–09 h 00 min and 35 mg/kg at 16 h 00 min–18 h 00 min, for 3 days after implantation of the morphine pellet. Behaviour indicating abstinence was observed for 10 min after injection of naloxone hydrochloride 0·2 mg/kg i.p.

Discussion

Lewis (1923) first noted that adrenalectomy increases the susceptibility of rats to the lethal effects of morphine. Subsequent studies (Scott, 1923; MacKay & MacKay, 1929) confirmed these findings and indicated that the lethal dose of morphine in adrenalectomized rats was approximately 1/4 to 1/5 of the lethal dose in sham-operated controls. In contrast to these findings, Puharich & Goetzl (1947) and Friend & Harris (1948) reported that adrenalectomy increased resistance of

rats to the antinociceptive effect of morphine, when analgesia was assessed by the tail-clamp procedure. The criterion for pain in the tail-clamp procedure was vocalization. In the present investigation, the AD50 of morphine was reduced in adrenalectomized rats when measured by the tail-flick procedure of D'Amour & Smith (1941); a result similar to that obtained by Miller, George, Elliot, Sung & Way (1955) and by Gebhart & Mitchell (1972). It is conceivable that adrenalectomy may increase susceptibility to the lethal effects of morphine, decrease the dose of morphine required to inhibit the response to thermal stimuli and yet raise the dose of morphine required to inhibit vocalization evoked by tail-clamping. The limitations of the experimental methods for assessing pain in animals appear to preclude a definite answer to the question of whether morphine is more or less potent in reducing pain after adrenalectomy.

The role of the adrenal hormones in morphine analgesia is still unclear. Puharich & Goetzl (1947) suggested that adrenaline, released from the adrenal medulla by morphine, was responsible for the antinociceptive action of morphine. Miller *et al.* (1955) and Paré (1969), however, concluded that large doses of adrenaline raised the threshold of rodents to aversive stimulation mainly through general motor impairment and debilitation. It is therefore uncertain if the observed alterations in behaviour which may have been caused by release of adrenaline, are in fact due to analgesia. Adrenocortical hormones in pharmacological doses also modify morphine analgesia (Winter & Flataker, 1951) but it is not known if *in vivo* release of these hormones significantly affects the actions of morphine. It should be noted that Tsou & Jang (1964) and Herz, Albus, Metys, Schubert & Teschemacher (1970) demonstrated that analgesia can be produced by application of morphine to discrete areas of the brain. Thus the mechanisms of morphine analgesia appear to be independent of hormonal discharges from the adrenal glands.

Development of tolerance to morphine in adrenalectomized rats has been reported by Way *et al.* (1954) and by Gebhart & Mitchell (1972). The results in this investigation confirm the conclusions of these authors that the adrenals are not necessary for the development of morphine tolerance. Tanabe & Cafruny (1958) noted that the course and intensity of morphine withdrawal in 6 hypophysectomized rats was similar to that of the controls. In the present investigation, removal of the adrenal glands or injections of corticosterone did not alter the intensity of the precipitated abstinence syndrome. It may be concluded that development of tolerance to and physical dependence on morphine is not significantly mediated by adaptive changes in the pituitary-adrenal axis.

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