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PLASMA CYCLIC AMP IN THE MORPHINE-TOLERANT RAT*

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Abstract—Effects of morphine on plasma cyclic AMP levels in the rat were investigated. Morphine (20 mg/kg, s.c. + 4 mg/kg/hr, i.v.) produced an increase in plasma cyclic AMP concentrations at 1 hr and sustained elevated levels for several hours, but the levels returned to normal levels at 48 hr. The 48 hr increase in plasma cyclic AMP elicited by morphine (20 mg/kg, s.c.) was far smaller than that of 1 hr; the plasma concentration of morphine was at the same level as the 1 hr. This indicated the development of tolerance to the morphine induced increase in plasma cyclic AMP. At 48 hr an epinephrine challenge increased plasma cyclic AMP to the same extent as in naive rats. Therefore, desensitization of peripheral tissues as being responsible for the increase in the plasma cyclic AMP could be ruled out. The development of tolerance by the sympatho-adrenal reflex elicited by morphine was suggested. In naloxone-precipitated abstinence, plasma cyclic AMP showed a 4-fold increase but, on spontaneous withdrawal, it showed on a slight but significant increase. It was concluded that plasma cyclic AMP is a sensitive index of tolerance to and dependence on morphine.

It has been suggested that plasma cyclic AMP serves as a good index of adrenergic activity, and has advantages over other markers including catecholamine metabolites and dopamine-β-hydroxylase [1]. Recently we have found that plasma cyclic AMP was increased by morphine administration to naive mice and by spontaneous withdrawal or naloxone-precipitated abstinence [2, 3] in the species. These results are consistent with the previous view that catecholamines may be released from the adrenal glands under these conditions [4–7].

It remains controversial whether tolerance develops in reaction to the morphine-elicited stimulation of the sympatho-adrenal axis. Some investigators observed that the development of tolerance to the analgesic effect of morphine was accompanied by a tolerance to the reflex sympatho-adrenal discharge. This was understood by measuring urinary excretion of catecholamines [4, 5, 8], adrenal catecholamine contents [9] and epinephrine concentration in adrenal vein blood [10]. Other authors [11] did not support that hypothesis after analyzing catecholamine contents, tyrosine hydrolase and dopamine β-hydroxylase activities in the adrenals. Measurement of plasma catecholamines may provide a direct answer to the question. However, plasma catecholamine concentrations reflect, too sensitively, the stress of laboratory manipulations associated with the methods of blood collection rather than specific pharmacological treatments [6, 12].

Therefore, by measuring plasma cyclic AMP levels during continuous intravenous infusion of morphine into rats, we examined the hypothesis of tolerance development in the sympatho-adrenal axis. The validity of plasma cyclic AMP as a biochemical index of tolerance to and dependence on morphine will be discussed.

MATERIALS AND METHODS

Animals. Male Wistar rats with 250–350 g had free access to food and water, and were maintained under controlled environmental conditions, with a 12 hr light-dark cycle in a room with controlled temperature (22 ± 1°).

Administration of morphine. Animals were cannulated in the right jugular vein under light ether anesthesia [13], and were infused intravenously with saline for control and with morphine for treatment groups as described previously [14]. At time 0, morphine (20 mg/kg, s.c.) was injected, and additionally infused (4 mg/kg/hr, i.v.) for 48 hr. Again at 48 hr, the same dose of morphine (20 mg/kg, s.c.) was administered. The purpose of this subcutaneous administration was to (1) more rapidly induce tolerance and dependence, and (2) make comparison of the effect of morphine between naive and morphine-tolerant conditions.

Cessation of the infusion and abstinence. Abstinence was precipitated with naloxone (5 mg/kg, s.c.), which was injected 30 min after the cessation of infusion.

The assay of morphine, cyclic AMP and glucose in plasma. Plasma levels of morphine were determined by a fluorometric method [15], as described earlier [14]. Plasma cyclic AMP was quantitated as described elsewhere [2, 3]. In brief, a portion (50 μl) of blood samples was mixed with 150 μl of saline supplemented with 10 mM EDTA (pH 7.4). After centrifugation at 2000 × *g* for 30 min at 4°, the supernatant was directly analyzed for cyclic AMP by the radioimmunoassay developed by Honma *et al.* [16]. The plasma glucose was estimated by a kit from

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Boehringer Mannheim Co. (West Germany), based on the glucose oxidase procedure [17].

Chemicals. The following drugs were used: morphine hydrochloride (Sankyo Co. Ltd., Tokyo, Japan), naloxone hydrochloride (Endo Labs. Inc., New York, NY) and epinephrine bitartrate (Nakarai Chemicals Co., Kyoto, Japan). All doses were calculated as the salt. All drugs administered were dissolved in physiological saline, unless otherwise stated.

Data analysis. For comparison of pharmacokinetic parameters of morphine between spontaneous withdrawal and naloxone-precipitated abstinence, a simpler one-compartment model was chosen [18].

Results of plasma concentrations of cyclic AMP and glucose were analyzed by the two tailed *t*-test, unless otherwise stated.

RESULTS

Effect of morphine administration on plasma cyclic AMP and glucose

Patrik *et al.* [15] showed that a single injection of morphine (16 mg/kg, s.c.) caused peak concentration at around 50 min. In our study (Fig. 1), the highest concentration occurred at 1 hr by a single injection and intravenous infusion. These results indicate that at least for 1 hr during the initial period of infusion, the plasma levels of morphine were maintained chiefly by the subcutaneous injection. The steady-state concentrations were about 4 μg/ml. Plasma cyclic AMP concentrations increased at 0.5 hr and sustained elevated levels for several hours. This increase in plasma cyclic AMP was not due to the stress associated with the infusion *per se*, because rats infused with saline did not show the elevation of plasma cyclic AMP levels (data not shown). However, the levels returned to basal levels at 48 hr. Plasma glucose concentrations altered in parallel with those of morphine, although the elevated peak at 1 hr values were not evident. The second injection of morphine (20 mg/kg, s.c.) at 48 hr did not produce a significant increase in plasma cyclic AMP and glucose, while plasma concentrations of morphine increased to the same level realized by the initial morphine challenge (see closed arrows in Fig. 1). These results suggest that the increase in plasma cyclic AMP develops a tolerance to morphine.

Effect of epinephrine on plasma cyclic AMP levels in naive and morphine-dependent rats

Epinephrine injection (i.p.) to naive rats produced an increase in plasma cyclic AMP in a dose-dependent manner (Fig. 2). The same degree of increase was produced by the drug injection into the morphine-dependent rats. The dose of 500 μg/kg was considered to produce an almost maximal increase in plasma cyclic AMP, since the same dose of isoproterenol had the maximal effect on plasma cyclic AMP levels [1]. These results here rule out the development of tolerence to epinephrine by the β-adrenoceptors responsible for increasing plasma cyclic AMP levels in the morphine-dependent rats.

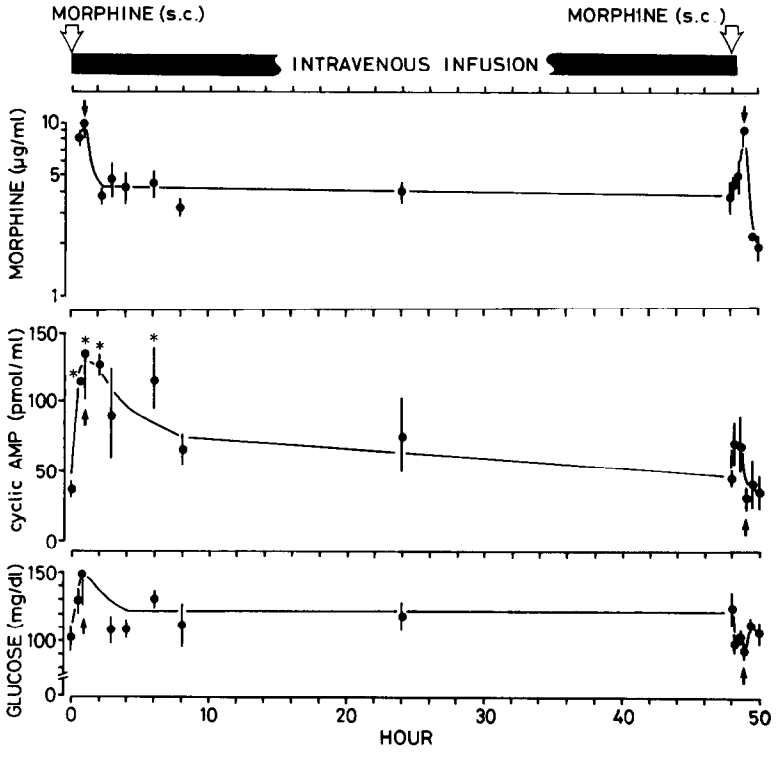

Fig. 1. Time course of plasma concentrations of morphine, cyclic AMP and glucose. Morphine was infused intravenously at a rate of 4 mg/kg/hr for 48 hr and was also injected (20 mg/kg, s.c.) at time 0 and at 48 hr. Points and bars represent the means ± S.E.M. of 3 to 11 rats. The open arrows show the time of subcutanous injection of morphine. The smaller and closed arrows show the values at 1 hr after each injection of morphine. * $P < 0.01$ vs time 0 an $P < 0.01$ vs 48 hr.

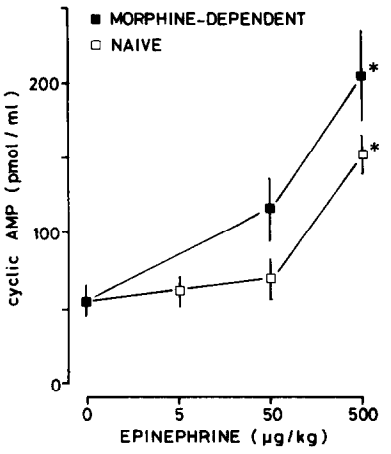

Fig. 2. Effect of epinephrine on plasma cyclic AMP in naive or morphine-dependent rats. Rats were made morphine-dependent by continuous intravenous infusion of morphine at a rate of 4 mg/kg/hr for 48 hr. Morphine (20 mg/kg, s.c.) was injected at time 0. At the 48 hr of the infusion, epinephrine (i.p.) was injected; 20 min after the injection, the infusion of morphine ceased and plasma cyclic AMP levels were determined. Epinephrine was dissolved in 0.01 per cent ascorbic acid and 0.9 per cent NaCl. Points and bars represent the means ± S.E.M. of 4 rats. * $P < 0.05$ vs 0 μg/kg of epinephrine. The differences between the 2 groups given the same dose of epinephrine are not statistically significant.

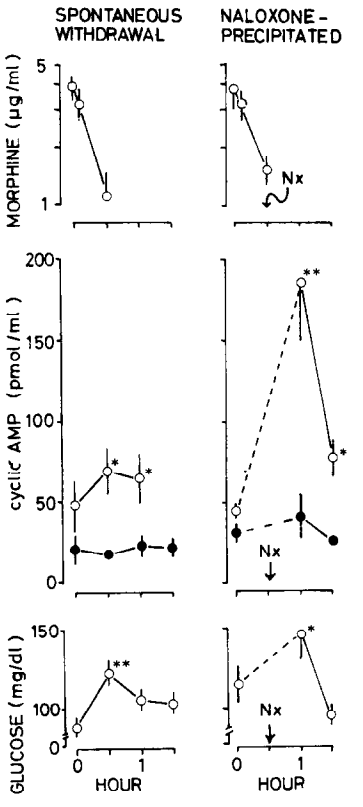

Fig. 3. Effect of withdrawal and naloxone-precipitated abstinence on cyclic AMP and glucose. Rats were infused intravenously for 48 hr with saline (0.23 ml/hr) (●) or morphine (4 mg/kg/hr) (○). Morphine (20 mg/kg, s.c.) was injected at time 0. Thirty minutes after the cessation of the infusion, naloxone (5 mg/kg, s.c.) was injected to elicit naloxone-precipitated abstinence and saline (5 mg/kg, s.c.) to induce spontaneous withdrawal. In the case of spontaneous withdrawal, blood samples were obtained serially from the same rats and a statistical analysis done with a paired *t*-test. Points and bars represent the means ± S.E.M. of 3 to 5 rats. * $P < 0.05$ vs 0 hr. ** $P < 0.01$ vs 0 hr. Nx: naloxone.

Effect of withdrawal and naloxone-precipitated abstinence on plasma levels of cyclic AMP and glucose

In naloxone-precipitated abstinence, plasma cyclic AMP showed a 4-fold increase, and at 1.5 hr returned toward the level of the end point of the infusion period, in contrast to the results observed in the spontaneous withdrawal group (Fig. 3). However, pharmacokinetic parameters were not different between spontaneous withdrawal and naloxone-precipitated abstinence in the apparent volume distribution (0.51 ± 0.12 vs 0.58 ± 0.11 l/kg) or in the biologic half-life (0.33 ± 0.03 vs 0.40 ± 0.07 hr). This effect of naloxone challenge on plasma cyclic AMP levels was not caused by naloxone *per se*, because naloxone injection into saline-infused rats did not produce any increase in plasma cyclic AMP concentrations (Fig. 3). These observations essentially confirms our previous results obtained from mice [3]. Plasma glucose concentrations increased in both groups.

DISCUSSION

Development of tolerance to sympatho-adrenal axis

The brain cyclic AMP is involved in the mechanisms of analgesic effect [19, 20] and tolerance and dependence [21]. The significant role of the cyclic AMP system in tolerance to and dependence on morphine has been widely reviewed by Collier [22]. However, the morphine-induced increase in plasma cyclic AMP that we observed is due to a somewhat different mechanism from those cited above in that we witnessed a catecholamine release from the adrenals [2]. In this study we found that plasma cyclic AMP levels were elevated in the initial phase, and returned to the normal level during the late period of infusion. The initial increase in plasma cyclic AMP at 1 hr may be due to the subcutaneous administration at time 0. The increase after 1 hr may be due to the continuous infusion. In any event, the second subcutaneous challenge of the same dose did not produce an increase in plasma cyclic AMP at 1 hr after the injection, despite the fact that plasma morphine levels were almost identical (Fig. 1).

It is well known that adenylate cyclase systems become refractory to β-adrenoceptor stimulants after a transient increase in tissue cyclic AMP [23]. However, there are no reports on such refractoriness of skeletal muscle, which may be the factor most contributive to plasma cyclic AMP increase induced by epinephrine [24]. The tolerance observed here may not be due to desensitization of peripheral tissues, since it was earlier reported [1] that isoproterenol increased the plasma cyclic AMP in rats that had been previously treated with isoproterenol to the same degree as the first injection. Furthermore, our results (Fig. 2) show that plasma cyclic AMP could

still be increased by epinephrine challenge in morphine-dependent rats to the same extent as in naive rats.

Failure to achieve an increase in plasma cyclic AMP during the late periods of infusion may not be due to the catecholamine depletion of the adrenal; Gunne [5] showed that when the same dose had been repeated for some days, the excretion of catecholamines returned toward normal levels. The cause of decreased urinary excretion of epinephrine and norepinephrine during chronic treatment of morphine could not be attributed to the depletion of adrenal catecholamines, because the content of catecholamine in the adrenal glands returned to normal after repeated administration of morphine [5, 9]. Our results (Fig. 3) show that plasma cyclic AMP was increased by naloxone-precipitated abstinence to even higher levels than the increase in plasma cyclic AMP at the early periods of infusion, which might not have occurred if the adrenal catecholamines had been depleted. These results suggest the development of tolerance to morphine of the sympatho-adrenal reflex.

Plasma cyclic AMP as a biochemical index of physical dependence

In accordance with our previous reports on mice [3], the present work shows that plasma cyclic AMP was clearly increased by spontaneous withdrawal and naloxone-precipitated abstinence in rats.

Based on the increase in plasma cyclic AMP (Fig. 3), we suggest that catecholamines are released from the adrenals in both withdrawal state and abstinence from morphine, consistent with the previous reports [4–7], although it remains an open question whether the effect of morphine withdrawal or abstinence is due to the same mechanisms as that of non-specific stress on the sympatho-adrenal axis *via* the central nervous system [25].

Changes in blood glucose concentrations during and after the infusion

The release of catecholamines from the adrenals is primarily responsible for morphine-elicited hyperglycemia [26]. Altszuler *et al.* [27] showed that hyperglycemia was sustained by the continuous intravenous infusion of epinephrine into the dog. There are no lines of evidence so far available, which show the development of tolerance to catecholamines-induced hyperglycemia. Thus, if epenephrine was released continuously for 48 hr from the adrenals, the blood glucose concentrations should have been maintained at higher levels throughout the infusion. Although hyperglycemia was not evident in this work, the results of blood glucose may be consistent with the view that (1) plasma catecholamines increase after morphine administration and return to normal levels during the morphine infusion and (2) increase again by spontaneous withdrawal or naloxone-precipitated abstinence.

We conclude that (1) tolerance of the sympatho-adrenal reflex elicited by morphine does develop and (2) that plasma cyclic AMP is a sensitive index of tolerance to and dependence on morphine in rats as well as in mice.

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