Rats were made tolerant to morphine in the following manner: the initial dose of 20 mg/kg by subcutaneous injection, twice daily, was gradually increased to 200 mg/kg, twice daily, in 7 weeks.

A subcutaneous administration of 20 mg/kg to nontreated rats produced a large slow waves in the MC, HPC and AMY within 15 minutes and it lasted for about 3.5 hours.

After repeated administration of morphine for 7 days, the onset of morphine-induced slow waves became larger and its duration became shorter than those observed in the nontreated rats. These tendencies increased with the lapse of time and on day 22 the slow wave-producing action of morphine was lost (Fig. 1).

The frequency of paradoxical sleep which is characterized by the arousal pattern in EEG, facial muscle twitch, respiratory irregularity and rapid eye movement was once per hour or less in daytime. Its frequency increased to several times from day 8 and thereafter did not change.

After an administration of nalorphine (1 mg/kg s.c.) at 2 weeks, the abstinence syndrome, such as restlessness, diarrhea and salivation etc, and the arousal pattern in EEG were observed. At 4 weeks, an administration of nalorphine produced the cataleptic syndrome and the spike formation which was located in HPC. The generation of spike formation was associated with that of cataleptic syndrome.

Present results indicate that electroencephalographic technique is a useful tool for testing morphine dependence in rat.

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CALCIUM-DEPENDENT HISTAMINE RELEASE BY ATP FROM ISOLATED RAT MAST CELLS

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Although there are evidences suggesting that an energy-requiring process is involved in the cellular reaction leading to the release of histamine from mast cells (1-3), the mechanisms of the "active" histamine release is not known. In an attempt to study the mechanisms of action of histamine liberators, we have tried to gain an insight into the ATPase activity of mast cells in the release of histamine, and found unexpectedly that adenosine-5'-triphosphate (ATP) itself induced morphological change of rat peritoneal mast cells, and in the presence of Ca^{2+} this was accompanied by the release of histamine. During this work we noticed a similar work by Diamant and Krüger (4), which in some respects agrees with our present results.

Mast cells were isolated from the rat peritoneal washing following arabic gum density gradient centrifugation, a slight modification of Kimura's procedure (5), and suspended in a buffered isotonic saline solution containing a small quantity of bovine serum albumin, with or without Ca^{2+} , and incubated at 37°C. The

FIG. 1. Effect of Ca^{2+} on histamine release by adenosine triphosphate. Each vessel contained, in a final volume of 2 ml: mast cells $1-3 \times 10^5$ cells/ml, 154 mM NaCl, 0.05% bovine serum albumin, 10 mM Tris-HCl buffer (pH 7.3). After incubation at 37°C for 10 minutes, the reaction was stopped by cooling in the ice bath, histamine in the supernatant and precipitate was determined. 6 experiments, mean $\pm$ S.E.

release of histamine from mast cells was stimulated on addition of ATP (0.01–0.5 mM, maximum at 0.075 mM) in the presence of Ca^{2+} (0.1 mM), while Ca^{2+} itself could not evoke histamine release (Fig. 1). Mast cells showed a drastic morphological alteration characterized by a marked swelling with extracellular detrusion of granules. With 0.1 mM ATP the histamine release was increased quantitatively by increasing concentration of Ca^{2+} up to 1.0 mM. In the Ca^{2+} -absent medium, ATP led to some morphological changes (swelling without degranulation) of mast cells, but there was no substantial increase of histamine release (Fig. 1). ATP could not be substituted by any of inosine-5'-triphosphate, guanosine-5'-triphosphate, uridine-5'-triphosphate and cytidine-5'-triphosphate. Adenosine-5'-diphosphate, adenosine-5'-monophosphate, adenosine, adenine and pyrophosphate were also ineffective. For the histamine release by ATP, Ba^{2+} and Sr^{2+} were less effective than Ca^{2+} , and other cations, such as K^+ , Mn^{2+} , Mg^{2+} , Fe^{2+} and Zn^{2+} were ineffective. Therefore, Ca^{2+} is essential for the histamine release by ATP, in contrast to a similar effect of compound 48/80 or sinomenine which caused histamine release in the absence of Ca^{2+} (2, 3, 6). EDTA inhibited the release of histamine by ATP in the presence of Ca^{2+} but it rather enhanced the swelling of mast cells induced by ATP alone. The histamine release by ATP in the presence of Ca^{2+} was completed within 10 minutes or so, whereas that by basic liberators finished within one minute at longest (7). The pH optimum for the histamine release was 7.1 and the temperature optimum, 37°C . SH-inhibitors, heavy metal ions, KCN and 2,4-dinitrophenol inhibited the release of histamine. Phase contrast- and electron microscopic observations revealed differences in the morphological change of mast cells between basic liberators and ATP with Ca^{2+} . These observations indicate that the histamine release by ATP differs in some aspects from that by basic liberators.

Further investigations are in progress on the mechanisms of histamine release induced by ATP and Ca^{2+} .

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