LACK OF ACTIVE SITE FOR KYOTORPHIN IN RAT MAST CELLS: ASSESSMENT BY ITS EFFECT ON IgE-MEDIATED $^{14}$C-SEROTONIN RELEASE

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Kyotorphin (KTP, Tyr-Arg), one of the endogenous analgesic opioid peptides, has been isolated from bovine brain by means of a sensitive analgesic method and reported to release Met-enkephalin (1). Recent electrophysiological studies, however, suggest that KTP acts on rat cerebral neurons through two different mechanisms: an enkephalin-releasing mechanism and a mechanism without involvement of enkephalin (2).

Immunological mediator release from rat mast cells is modulated by various neuroactive substances. By their effects on this immunological release, rat mast cells have been found to contain several types of pharmacological receptors including muscarinic (3), adenosine (4), PGE$_1$ (3, 5, 6), PGI$_2$ (7) and opioid receptors (5, 6). The fact that rat mast cells contain opioid receptors, taken together with the possibility that KTP may act on neurons directly as well as indirectly, prompted us to examine the effect of KTP on IgE-mediated $^{14}$C-serotonin release from rat mast cells.

Serosal mast cells from 5 rats of the Wistar strain (180–220 g) in each experiment were incubated at 37°C for 60 min in a 5-fold dilution of anti-ovalbumin IgE-rich serum of immunized BALB/c mice, containing 4 $\mu$g $^{14}$C-serotonin (0.1 $\mu$Ci/$\mu$g). After washing three times with mast cell medium (MCM: 154 mM NaCl, 2.7 mM KCl, 0.9 mM CaCl$_2$, 0.45 mM MgCl$_2$, 10% (V/V) Sørensen phosphate buffer (pH 7.0), 0.1% (W/V) gelatin and 0.1% (W/V) glucose), immunological activation of mast cells with ovalbumin (final concentration of 2 $\mu$g/ml) was carried out in MCM containing 5 $\mu$M serotonin, following preincubation at 37°C for 6 min with KTP, opiates, PGE$_1$ or a combination of KTP, opiates and PGE$_1$. Naloxone was added 2 min before preincubation. Antigen-induced $^{14}$C-serotonin release from rat mast cells was stopped 1 min after the antigen challenge by the formaldehyde method (5, 6). By this time, over 90% of the release reaction was completed (5, 6). After centrifugation, aliquots of the supernatant and pellet were taken for the determination of radioactivity with a liquid scintillation spectrometer. Reagents and their sources were as follows: Ovalbumin (Grade V), prostaglandin E$_1$, serotonin creatinine sulfate from the Sigma Chemical Company; levorphanol tartrate and dextrorphan tartrate from Hoffman-LaRoche; naloxone hydrochloride from Endo Laboratories Inc.; morphine hydrochloride from the Sankyo Co.; (side chain 2-$^{14}$C-)5-hydroxytryptamine creatinine sulfate (58 mCi/mmol) from New England Nuclear. Net antigen-induced release of $^{14}$C-serotonin, corrected for spontaneous release from control cells, was expressed as percent of $^{14}$C-serotonin release.

Various pharmacological agents are known to modulate mediator release from rat mast
cells. In general, an increase in the level of cellular cyclic AMP in these cells supresses and a decrease enhances mediator release (3). Stimulation of muscarinic receptors lowers the mast cell cyclic AMP content and thus potentiates compound 48/80-induced histamine release (3). Since mammalian cerebral neurons show excitatory response to iontophoretically applied acetylcholine through muscarinic receptors (8) as well as to KTP applied in the same way (2), we examined the effect of KTP on IgE-mediated 14C-serotonin release from rat mast cells. As shown in Fig. 1, however, KTP did not change the antigen-induced 14C-serotonin release from cells at concentrations of 10^-8 to 10^-4 M. In addition, KTP (10^-4 M) did not release 14C-serotonin from rat mast cells (not shown).

Previous experiments demonstrated that PGE1 (2X10^-8-2X10^-5 M) inhibits IgE-

Fig. 1. Effect of KTP on IgE-mediated 14C-serotonin release from rat mast cells. Inhibition is expressed as percent when 14C-serotonin release without KTP is taken as 100%. Values are the mean±S.E.M. (n=8). Net antigen-induced 14C-serotonin release was 24.9±3.8% (n=8), while spontaneous release was 5.1±0.3% (n=8) 1 min after antigen challenge.

Fig. 2. Reversal by opiates and KTP of PGE1 (2X10^-6 M)-induced inhibition of IgE-mediated 14C-serotonin release from rat mast cells. Inhibition of antigen-induced 14C-serotonin release by various agents is expressed as a percent when 14C-serotonin release by antigen alone is taken as 100%. Values are the mean±S.E.M. (n=8). Net antigen-induced 14C-serotonin release was 23.5±4.5% (n=8), while spontaneous release was 4.5±0.5% (n=8) 1 min after antigen challenge. The degree of inhibition achieved with 2X10^-6 M PGE1 was 38.5±4.0% (n=8). A, morphine; B, levorphanol; C, dextrorphan; D, KTP; MOR, morphine; LEV, levorphanol; NAL, naloxone.
mediated 14C-serotonin release in a dose-related manner and morphine (3 x 10^-7 to 3 x 10^-5 M) dose-dependently reverses this inhibitory response of mast cells to 2 x 10^-6 M PGE1. Moreover, this anti-PGE1 action of morphine was antagonized by naloxone which itself did not affect the inhibition by 2 x 10^-6 M PGE1 of this immunological reaction. In addition, the reversal by morphine of 2 x 10^-6 M PGE1-induced inhibition of mediator release could be mimicked by levorphanol (3 x 10^-8 to 10^-6 M), a congener of morphine, dose-dependently; but dextrorphan, an enantiomer of levorphanol, did not reverse this PGE1 effect at concentrations up to 10^-4 M. These observations suggest that rat mast cells contain opioid receptors (5, 6). All these observations were also confirmed in the present studies (Fig. 2). Since rat mast cells may contain opioid receptors, we also examined the interaction of 2 x 10^-6 M PGE1 and various concentrations of KTP on these cells by their effect on this release reaction. In contrast to the opiates, KTP did not show such anti-PGE1 action at concentrations of 10^-7 M to 10^-4 M (Fig. 2). With respect to IgE-mediated 14C-serotonin release itself, morphine (3 x 10^-5 M), naloxone (2 x 10^-4 M), levorphanol (10^-6 M) and dextrorphan (10^-4 M) caused no changes (not shown).

Thus, we could not demonstrate that rat mast cells have an active site for KTP despite the presence of the opioid receptors as demonstrated in rat brain homogenates (1). In conclusion, KTP does not modulate IgE-mediated 14C-serotonin release from rat mast cells.

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