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Phosphorylation of a single mast cell protein in response to drugs that inhibit secretion

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We have reported previously that the antiasthmatic drug cromolyn promotes the incorporation of radioactive phosphate into a single mast cell protein of apparent molecular weight of 78,000 [1], and we have suggested that this may provide a clue to the inhibitory effect of cromolyn on mast cell secretion. Here we report that some other agents known to inhibit mast cell secretion likewise increase the incorporation of radioactive phosphate into the 78,000 dalton protein band. Moreover, we find that one of these drugs, the recently synthesized compound Ro 21-7634, which is more effective than cromolyn in inhibiting mast cell secretion [2], is also a more potent inducer of phosphorylation of this 78,000 dalton protein.

Purified rat peritoneal mast cells [3] were preincubated in Locke's solution with $^{32}P_i$ for 1 hr at 37°. They were then washed and resuspended in Locke's solution [4], and 0.2-ml samples were incubated for 1 min at 37° with or without either Ro 21-7634 [2], cromolyn [1], quercetin or kaempferol [5], all of which are drugs that have been shown previously to inhibit mast cell secretion. At the end of the incubation period, all samples were dissolved in sodium dodecylsulfate, boiled for 5 min, and subjected to polyacrylamide gel electrophoresis and autoradiography as described [6].

Addition of Ro 21-7634 (0.1 μM) for 1 min at 37° clearly increased the incorporation of radioactive phosphate into a protein band with an apparent molecular weight of 78,000 (Fig. 1, compare lanes 1 and 2). This effect is qualitatively similar to that reported previously for cromolyn [1]. Cromolyn, however, in the same concentration (0.1 μM) only minimally increased the incorporation of radioactive phosphate into the 78,000 dalton protein band, and an effect comparable with that shown with Ro 21-7634 could only be achieved with a 100-fold higher concentration (10 μM) (Fig. 1). Quercetin and kaempferol, two other drugs reported to inhibit mast cell secretion [5], also increased the incorporation of radioactive phosphate into a single protein band with an apparent molecular weight of 78,000 when tested at the single concentration of 10 μM (data not shown).

Simultaneous addition of Ro 21-7634 (7.57 μM) along with 48/80 (1 μg/ml) for 1 min at 37° did not increase the incorporation of radioactive phosphate into the 78,000 dalton protein band above that seen with either compound alone. The phosphorylation of the other three protein bands (68,000, 59,000 and 42,000 daltons) affected by 48/80 was not altered by the simultaneous addition of Ro 21-7634 (data not shown). Results similar to those obtained with Ro 21-7634 were noted previously with cromolyn [1].

The previous suggestion that phosphorylation of a single 78,000 dalton protein in response to cromolyn may be closely associated with the ability of this drug to inhibit histamine release is strengthened by the present observation showing that a more potent antiallergic drug, Ro 21-7634, produces the same effect in lower concentrations. The additional findings that quercetin and kaempferol, two other antiallergic agents, had qualitatively similar effects indicate that the ability to stimulate phosphorylation of a 78,000 dalton protein is a property shared by several antiallergic agents and may be a common component of their mechanism of action.

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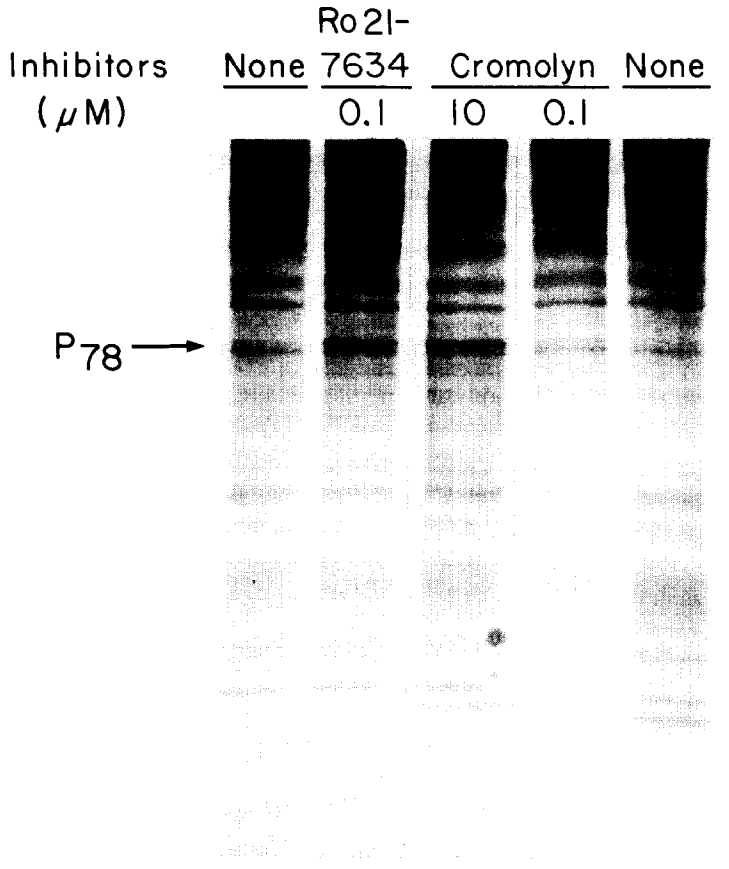

Fig. 1. Autoradiograph comparing the effects of Ro 21-7634 and cromolyn on the incorporation of radioactive phosphate into the 78,000 dalton protein band of intact mast cells. Mast cells were collected from fifteen male Sprague–Dawley rats (400 g, Charles River Breeding Laboratories, Wilmington, MA) and were purified (90 per cent purity) as described previously [3]. They were then preincubated in 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes)-buffered Locke's solution [1] with 0.75 mCi of carrier-free $^{32}P_i$-labeled inorganic phosphate (9120 Ci/nmole, New England Nuclear Corp., Boston, MA) per ml for 1 hr at 37°. The cells were then washed three times by centrifugation at 180 *g* for 5 min at 4° and resuspended in 5 ml of cold Locke's solution. Mast cell suspensions (0.2 ml) were preincubated in Locke's solution at 37° in plastic tubes for 5 min following which cromolyn (0.1 and 10 μM, Fisons, Bedford, MA) or Ro 21-7634 (0.1 μM, Hoffmann-La Roche, Nutley, NJ) was added in 0.1 ml of Locke's solution. After incubation, for 1 min at 37°, 150 μl of a stop solution [10% sodium dodecylsulfate (SDS, w/v), 100 mM Tris–HCl (pH 7.4), 5 mM β-mercaptoethanol, 0.1 g/ml sucrose, and 0.02 mg/ml bromophenol blue tracking dye] was added, the solutions were boiled for 5 min, and the proteins were analyzed by electrophoresis on 10% polyacrylamide gel and autoradiography [6].