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Somatostatin-induced phosphorylation of mast cell proteins

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The tetradecapeptide somatostatin, which was first isolated from brain and characterized by its ability to inhibit secretion of growth hormone from the pituitary gland [1, 2], is now known to be present in cells of diverse type and to inhibit secretion from various secretory cells [3]. Remarkably, however, somatostatin has quite the opposite effect on mast cells in which it strongly stimulates secretion [4, 5]. This stimulant effect resembles that of the familiar mast cell secretagogue compound 48/80 and of the ionophore A23187 inasmuch as it involves exocytosis and is dependent on energy and calcium [5, 6]. The latter two secretagogues have been found recently to cause a prompt increase in the incorporation of radioactive phosphate into three specific mast cell proteins of apparent molecular weights of 42,000, 59,000 and 68,000, from which it has been concluded that such phosphorylation may be involved in stimulus-secretion coupling [7]. With 48/80, an additional protein band with an apparent molecular weight of 78,000 showed a relatively slowly developing incorporation of radioactive phosphate that reached a peak only after about 60 sec when incorporation of radioactive phosphate into the other protein bands was already waning. Since later experiments [8] revealed that cromolyn, an inhibitor of mast cell secretion, causes a *selective* incorporation of radioactive phosphate into the 78,000 dalton band (as do other inhibitors: Ref. 9), the delayed phosphorylation of this same band seen following stimulation by 48/80 was suggested as perhaps having to do with termination of the effect [8]. Here we report that the secretagogue effect of somatostatin on mast cells involves the same pattern of protein phosphorylation noted with 48/80.

Purified rat peritoneal mast cells [5] were preincubated with $^{32}P_i$ for 1 hr at 37°, washed three times with Locke's solution [7], and resuspended in the same solution. Portions of the cell suspension were incubated for 1 min at 37° with or without somatostatin (5 μg/ml), 48/80 (1 μg/ml), or A23187 (1 μg/ml) in a total volume of 0.3 ml. At the end of the incubation period the cells were dissolved in sodium dodecylsulfate, boiled for 5 min, and subjected to polyacrylamide gel electrophoresis and autoradiography (see legend to Fig. 1).

Addition of somatostatin (5 μg/ml) for 1 min caused an increase in the incorporation of radioactive phosphate into four protein bands with apparent molecular weights of 78,000, 68,000, 59,000 and 42,000 (Fig. 1, compare lanes 1 and 2), and this pattern was identical with that seen with 48/80 (Fig. 1, lane 3). As reported before [7], A23187 supported the phosphorylation of all but the 78,000 dalton protein band (Fig. 1, lane 4). In this single respect, A23187 produced a pattern of phosphorylation different from that seen with either somatostatin or 48/80. The phosphorylation of several protein bands of low molecular weight was occasionally affected by the incubation conditions, but such effects were inconsistent.

The described stimulant effect of somatostatin on protein phosphorylation was observed five out of seven times, but was generally weaker than that of 48/80. This behavior may be related to the fact that when mast cells are purified and then used in the absence of bovine serum albumin—as was required by our phosphorylation experiments—their maximum secretory response requires longer incubation and higher doses when elicited by somatostatin than by 48/80 [6].

In showing that somatostatin, which stimulates mast cell secretion, produces in mast cells the same pattern of protein phosphorylation as does the classic secretagogue 48/80, the results fortify the conclusion [6] that the stimulant action of both drugs is similar. In addition, the results provide a further indication that protein phosphorylation is involved in stimulus–secretion coupling in mast cells. Finally, the results allow somatostatin to be added to the large [11] and growing [12] list of regulatory agents that affect the phosphorylation of specific proteins in their target cells.

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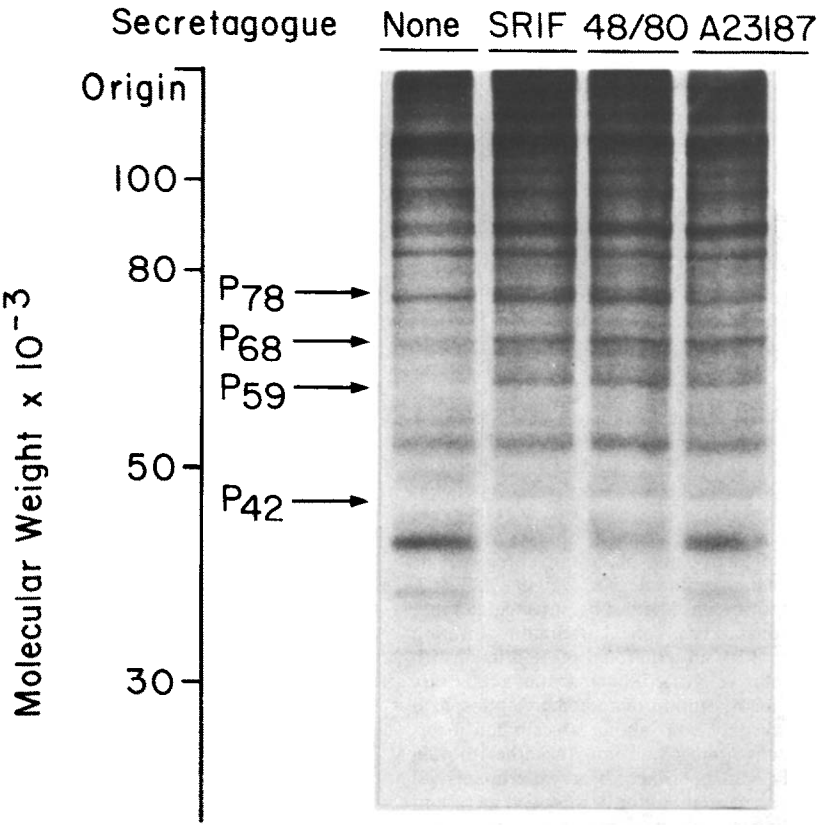

Fig. 1. Effects of somatostatin, 48/80, and A23187 on endogenous phosphorylation of mast cell proteins. Peritoneal mast cells were collected from fifteen male Sprague–Dawley rats (400 g, Charles River Breeding Laboratories, Wilmington, MA) and purified (90 per cent purity) as described previously [5]. They were then preincubated (1.5×10^6 cells/ml) in a 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes)-buffered Locke's solution [7] in the presence of 0.75 mCi/ml carrier-free $^{32}P_i$-labeled inorganic phosphate (NEN, 9120 Ci/mmole) 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 somatostatin (SRIF, 5 μg/ml, Bachem Torrance, CA), 48/80 (1 μg/ml, Burroughs Wellcome Co., Research Triangle Park, NC), or A23187 (1 μg/ml, Eli Lilly, Indianapolis, IN), was added in 0.1 ml of Locke's solution. After a 1-min incubation 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, and the tubes were placed in a boiling water bath for 5 min. Electrophoresis on 10% polyacrylamide gels, protein staining of the gels, and autoradiography were carried out as described previously [10]. The apparent molecular weights of the phosphorylated protein bands indicated by the arrows (78,000, P_{78}; 68,000, P_{68}; 59,000, P_{59}; and 42,000, P_{42}) were determined by calibrating the gel with standard proteins of known molecular weight. Degranulation, used as a measure of mast cell secretion, was monitored by light microscopy.