180°, and so on. These angles were measured to within 5° with an eyepiece protractor in an inverted microscope. From these angles, an average cosine for a particular population of eggs could be determined. An average cosine of +1 would mean that all rhizoids grew directly toward the fiber; −1 would mean that all rhizoids grew directly away from the fiber; and 0 would indicate no orientation.

Table 1 gives the average cosine as a function of the time between fertilization and placement of the cells near the fibers. If the eggs were placed near the fibers during the first 4 hours after fertilization, the average cosine was approximately +0.45. After that, the polarizing effect of the ionophore-coated fibers seemed to decline. Control eggs placed near uncoated fibers had an average cosine of almost zero. Similar experiments in which the potassium ionophore valinomycin was used produced little or no polarization. The results with A23187 were independent of *p*H from 8.1 to 6.5, and lowering the concentration of magnesium from 50 to 5 m*M* also had no effect.

The fraction of eggs that germinated was usually 10 to 20 percent smaller among eggs near the ionophore-coated fibers than among those farther away; however, in three cases, fewer than half of the eggs near the fibers germinated while all of the farther eggs germinated. In two of these cases, the average cosine was small but positive (+0.10, +0.16); in the third it was actually negative (−0.15). (These apparently aberrant cases are not included in the averages given in Table 1.) Another effect of proximity was that the morphology of rhizoids near the fibers was somewhat modified; they tended to be broader and shorter than those farther away (Fig. 2).

We examined the effect of A23187 on calcium influx in these eggs to verify that it was indeed acting as a calcium ionophore. Using methods described in (*7*), we found that the addition of A23187 to a nominal calcium concentration (5*M*) doubled the calcium influx from 0.1 to 0.2 pmole/cm²-sec.

To investigate whether the efflux of protons rather than the influx of calcium ions was responsible for the polarization of the eggs, we grew eggs on a Ronchi ruling (Edmund Scientific), a piece of glass that has alternate transparent and opaque strips, each 50 μm wide. The eggs were illuminated from beneath the Ronchi ruling by light that was passed through a red filter with a sharp absorption edge at 610 nm (Klinger Scientific, RG610). The intensity of the light reaching the cells was 1000 lm/m². Many eggs were approximately half illuminated by the red light. We reasoned that if a hydrogen ion gradient were maintained in the cytoplasm of the half-illuminated cells, the illuminated sides would be more alkaline than the dark sides, since photosynthesizing chloroplasts continuously take up CO_2. We observed that the zygotes were equally likely to germinate on either side. Of the 336 zygotes counted, 172 formed rhizoids on the shaded side and 164 on the illuminated side. These results are consistent with the finding that both unilateral red light and plane-polarized red light are ineffective in polarizing the zygotes (*8*, *9*). It seems unlikely, therefore, that the polarizing effects of the A23187 gradient are mediated by a transcytoplasmic hydrogen ion gradient.

Table 1. Polarization of *Pelvetia* zygotes by ionophore-coated fibers. At least 70 eggs were used in the determination of the average cosine in each experiment. Orientation measurements were made only of eggs whose bodies (the spherical portion excluding the rhizoidal outgrowth) were no farther than 100 μm from the fiber. All of these experiments were done with eggs in darkness in 10 m*M* tris-buffered natural seawater (*p*H 8.1, 15°C).

Fiber	Experiments (No.)	Time* (hours)	Cosine
A23187-coated	14	0–2	+0.45 ± 0.08
	10	2–4	+0.47 ± 0.19
	3	4–6	+0.31 ± 0.18
Uncoated	4	0–2	−0.01 ± 0.01
Valinomycin-coated	7	0–2	+0.07 ± 0.12

*Time between fertilization of eggs and placement of eggs near fibers.

In summary, we have shown that polarizing fucoid eggs grown near an A23187-coated glass fiber tend to form their rhizoids on the sides near the fiber. These results are consistent with the idea that the formation of an intracellular calcium gradient is a necessary step in the polarization process. We anticipate that the method used in this study will be applicable to the study of polarity and tropism in other cellular systems. Indeed, it has recently been shown (*10*) that the spores of the common moss *Funaria hygrometrica* respond to a gradient of A23187 by forming their rhizoids on the side of higher concentration.

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Placental β-Endorphin–Like Peptides

Abstract. *Acid extracts of human placental tissue contain, by both radioimmunoassay and radioreceptor assay, β-endorphin–like material. Half of this material will not go through a 5000-dalton filter and on Sephadex G-200 has a molecular size between 25,000 and 50,000 daltons. Of the material going through a 5000-dalton ultrafilter, 80 percent is excluded on Sephadex G-25 and held back, very slightly, on Bio-Rad P6, indicating a molecular size of approximately 4500 to 4800 daltons. Thus, placenta appears to have macromolecular precursors from which a β-endorphin–like material is released, with a size approximately 12 amino acids longer than that of the pituitary hormone.*

The endogenous pituitary peptide β-endorphin competes with morphine for the same binding sites in the brain (*1*). This peptide has the same amino acid sequence as the last 31 amino acids in β-lipotropin (61–91) and is an endogenous, morphine-like analgesic (*2*). Amnionic fluid has been shown (*3*) to contain a significant amount of β-endorphin as judged by radioimmunoassay (RIA). Further, acid extracts of human placenta have been shown to contain (by RIA) both β-endorphin and adrenocorticotropic hormone (ACTH) as well as β-lipotropin (*4*). Recently, evidence has been presented that there is in the placenta a common precursor of lipotropin and β-endorphin with a number of mole-

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cules weighing between 48,000 and 36,000 daltons (*5*).

Utilizing an RIA that was developed by the New England Nuclear Company with antiserums from rabbits prepared against synthetic human β-endorphin (synthesized by the Peptide Division of the Beckman Company) and the radioreceptor assay (RRA) involving the displacement of tritiated naloxone from binding sites in the brain after extensive washing to eliminate contaminating peptidases (*6*), we have studied human placenta-derived β-endorphin molecules. The binding of [^{3}H]naloxone to opiate receptors in the rat brain homogenate was as stereospecific as levorphanol and was 500 times more potent in displacing naloxone than its biologically inactive enantiomer dextrofan. The [^{3}H]naloxone binding was also inhibited by naltrexone, a potent narcotic antagonist, and by synthetic β-endorphin (Beckman). The crude placental extract required 4.4 mg/ml to displace 50 percent of the [^{3}H]naloxone.

Because β-endorphin is a peptide subject to proteolysis, we extracted the human placenta with dilute mineral acid (0.1*M* HCl, *p*H 1.8). This extract was clarified by centrifugation, quickly neutralized, and incubated at 70°C for 20 minutes to denature both peptidases and proteases. We have shown that human placenta contains cathepsin D protease activity, maximally active at *p*H 3 and completely inhibited by pepstatin. The resulting clarified supernatant in turn was expressed through a 5000-dalton hollow-fiber Amicon ultrafilter and concentrated over a 1000-dalton (UM-2) membrane filter. The retained material was assayed by RIA and RRA and then lyophilized. The salt-free preparation was then redissolved in elution buffer and subjected to molecular exclusion chromatography with Sephadex G-25. Of the total reactive material in three extracts from three separate placentas, approximately 60 percent of the RIA activity passed through the 5000-dalton ultrafilter. Of the 40 percent of RIA- and RRA-active material that did not pass through this filter, about 85 percent was found by Sephadex G-200 exclusion chromatography to be between 25,000 and 50,000 daltons, as has been suggested for placenta (*5*).

The elution profile from Sephadex G-25 chromatography is indicated in Fig. 1, where RIA activity is compared with the 280-nm absorbance of the column eluate and the elution volume of both ^{125}I-labeled synthetic pituitary endorphin (3400 daltons) and bacitracin (1411 daltons), which were used as standards for molecular size. The ^{125}I-endorphin was also chromatographed on a Sephadex G-25 column after being mixed with crude placental extract. The elution volume was the same as that demonstrated by pure standard.

Approximately 75 percent of the RIA-reactive β-endorphin was found in the void or exclusion volume of Sephadex G-25 (Fig. 1). This amount is significantly larger than that of the standard ^{125}I-labeled β-endorphin. About 20 percent of the total RIA activity that could be expressed through a 5000-dalton ultrafilter was in a position between β-endorphin and bacitracin, with a molecular size of approximately 2200 daltons. This corresponds to γ-endorphin in size; but γ-endorphin, which is released from β-endorphin by the proteolytic activity of cathepsin D and has the same sequence as the first 17 amino acids of β-endorphin, does not cross-react with antiserum to β-endorphin (*7*).

After RIA, the β-endorphin from the void volume was pooled, as was the RIA-identifiable material in the range of 2200 daltons. Samples from each pool were tested for their ability to displace radioactive naloxone from rat brain binding sites. The results indicated that approximately 80 percent of the total RIA activity that moved through the 5000-dalton filter was in the void volume and approximately 20 percent was held back behind standard β-endorphin. This distribution and size was unaffected regardless of whether the *p*H of the chromatographic columns was 7.5 or 4.5. The elution profile of this material was developed on a Bio-Rad P6 column, which has an exclusion limit of 6000 daltons. The results indicate that this RIA-determined β-endorphin-like activity was held back on P6 but behind the void volume; that is, its molecular size was slightly less than 5000 daltons.

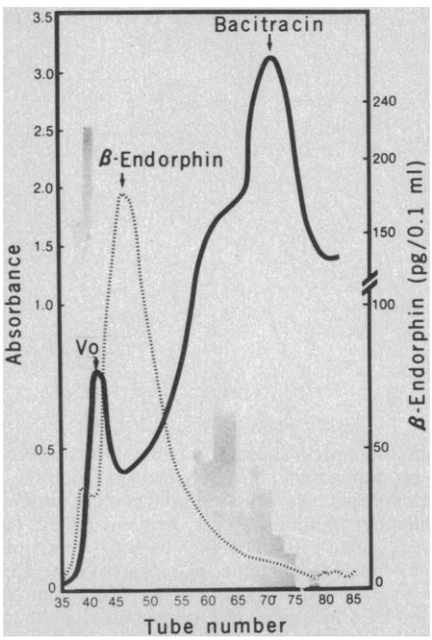

Fig. 1. Sephadex G-25 chromatographic elution pattern and the profile of β-endorphin immunoreactivity. The acid extract of the human placenta which passed through a 5000-dalton ultrafilter was chromatographed on a column (2.5 by 90 cm) in 10 m*M* sodium acetate buffer, *p*H 4.3, and 0.15*M* NaCl. The absorbance at 280 nm (solid line, left ordinate) is compared with the immunoreactivity determined by RIA (shaded areas, right ordinate). The exclusion volume (V_0) was determined with bovine serum albumin (68,000 daltons) and the column was standardized in addition by bacitracin (1411 daltons) and by ^{125}I-labeled synthetic human β-endorphin (3465 daltons).

Thus, by both RRA and RIA mineral acid extracts of human placenta from three patients undergoing natural childbirth contained two β-endorphin-like materials, most of which had approximately 12 more amino acids than the material characteristically extracted from the pituitary. The nature of the material of smaller molecular size is unclear, since, despite its size, it is unlikely to be (immunologically) γ-endorphin. It may represent a peptidase degradation product of the larger precursor which still possesses both RRA and RIA activity despite its relatively small molecular size. Thus human placenta contains two β-endorphin-like peptides, which differ markedly in size from the classical pituitary-derived hormone. Recently, it has been shown from complementary RNA sequence analyses of messenger RNA for β-lipotropin that its precursor has a much larger molecular size than 91 amino acids (*8*) and perhaps the enzymes which release placental β-endorphin from its macromolecular precursor hydrolyzed the peptide bond at a position where the 5000-dalton hormone can be made. Finally, extracts of liver, lung, thymus, spleen, and kidney were shown by both RIA and RRA to be devoid of β-endorphin activity.

The physiological function and possible clinical importance of placental endorphins has yet to be determined, although they may occur as a natural antidote for the pain and stress of parturition.

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Antiallergic Drug Cromolyn May Inhibit Histamine Secretion by Regulating Phosphorylation of a Mast Cell Protein

Abstract. *Cromolyn inhibited histamine release from mast cells that was induced by a classic secretagogue and correspondingly increased incorporation of radioactive phosphate into a 78,000-dalton protein. These effects on histamine secretion and on protein phosphorylation were rapid in onset and both showed tachyphylaxis. Cromolyn may therefore act by altering the phosphorylation of a protein involved in the regulation of secretion.*

Mast cells have been increasingly used as a model system for analysis of the cellular mechanisms involved in secretion (*1*) because they secrete by exocytosis, a mechanism common to many secretory cells (*2*), and, like most secretory cells, seemingly use calcium as a mediator in stimulus-secretion coupling (*3*). In addition, secretion by mast cells is of clinical interest because of its involvement in allergic diseases (*4*, *5*). In recent years it has been discovered that mast cell secretion can be inhibited by cromolyn sodium (cromoglycate) (*6*); this has led to a major advance in the prophylactic treatment of asthma (*7*).

Interest in discovering how cromolyn acts is intense, but little is known. It is evident that the action of cromolyn is not restricted to antigen-evoked secretion, for the drug can also potently inhibit, for example, mast cell secretion induced by the polyamine 48/80, the classic mast cell secretagogue (*8*). But further clues are sparse. Some authors (*6*) have speculated that cromolyn may be a "membrane stabilizer" and others (*9*) have suggested that it may block calcium entry. Here we present evidence that cromolyn may act to inhibit histamine release from mast cells by regulating phosphorylation of a specific mast cell protein. The experiments leading to this conclusion were prompted by the recent observation (*10*) that exposure of mast cells to 48/80 or the calcium ionophore A23187 alters the phosphorylation of several protein bands as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Since phosphorylation of three of these protein bands showed calcium dependence and occurred rapidly (while the secretory response was still developing), we suspected that these phosphorylation reactions might be involved in the induction of exocytosis and offer a clue to the still enigmatic role of calcium in stimulus-secretion coupling. In addition, the same experiments showed that a fourth protein band (78,000 daltons) incorporated radioactive phosphate later in the course of the response to 48/80, when secretion had largely run its course and the cells were presumably recovering. Here we report that exposure to cromolyn, whose effect is not to induce secretion but to inhibit it, results in selective phosphorylation of the same 78,000-dalton protein band. Together, these results suggest a possible association between phosphorylation of this particular protein band and the cellular mechanisms for physiological termination and pharmacological block of the secretory response.

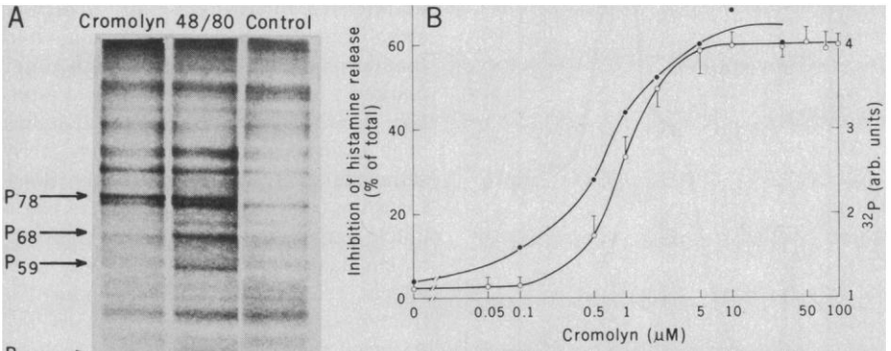

Fig. 1. (A) Autoradiograph showing the effect of cromolyn and of 48/80 on radioactive phosphate incorporation into proteins of intact mast cells. Mast cells (90 percent purity, 1.5×10^6 cells per milliliter) were first incubated in Hepes-buffered Locke's solution (*11*) with 0.75 mCi of carrier-free ^{32}P-labeled inorganic phosphate (9120 Ci/mmole; New England Nuclear) per milliliter for 1 hour at 37°C, and were then incubated (0.2 ml per tube) without any drug or with cromolyn (10 μM) or 48/80 (1 μg/ml) for 1 minute at 37°C as described in (*10*). The cells were then dissolved in a solution containing 10 percent (weight to volume) sodium dodecyl sulfate (*10*), placed in boiling water for 2 minutes, and analyzed for radioactive phosphate incorporation by electrophoresis on 10 percent polyacrylamide gels and by autoradiography (*19*). (B) Effect of cromolyn in various concentrations on the incorporation of radioactive phosphate into the 78,000-dalton protein band and on histamine secretion from rat peritoneal mast cells. Incorporation of radioactive phosphate (•) was determined by scanning autoradiographs of the type shown in (A) with a Canalco G-II microdensitometer. These results were obtained in the absence of 48/80. Similar dose-response curves were obtained in two other experiments: in both instances there was increasing phosphorylation of the 78,000-dalton protein band over the same range of cromolyn concentrations. To measure mast cell secretion (○), approximately 10^5 cells per milliliter were incubated simultaneously with 48/80 (1 μg/ml) and cromolyn (in the concentrations indicated) for 5 minutes at 37°C (*20*), and the histamine released was assayed fluorometrically in duplicate portions (*21*). Results represent mean ± standard error for five experiments.

Rat peritoneal mast cells were purified (90 percent) and incubated as described in (*11*). As a stimulus for histamine secretion, we used 48/80, which seemingly acts via receptors at the cell surface (*12*) to initiate exocytosis (*13*)—mainly by mobilizing cellular calcium (*14*). Mast cells which had been incubated for 1 hour with ^{32}P-labeled inorganic phosphate to label intracellular adenosine triphosphate (*10*), and then incubated with cromolyn for 60 seconds at 37°C, showed increased incorporation of radioactive phosphate into a single protein band with an apparent molecular weight of 78,000 (Fig. 1A, compare cromolyn and control lanes). This molecular weight is similar to that of the protein phosphorylated relatively late in the

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