Histamine, Norepinephrine, and Bradykinin Stimulation of Fibroblast Growth and Modification of Serotonin Response[1] (37714)

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Once the growth-enhancing property of serotonin on fibroblasts was known (1), the possibilities were raised that other biogenic amines or kinins have a similar effect or that these compounds might either antagonize or potentiate the effect of serotonin. To examine for these possibilities, fibroblasts were grown with histamine, norepinephrine, or bradykinin alone and in combination with serotonin. Prostaglandin E_2 was also tested.

The results indicate that serotonin and norepinephrine consistently enhance fibroblast growth while histamine has a lesser or inconstant effect. Distinctive dose–response curves for the three biogenic amines suggest different mechanisms of action. Bradykinin enhances and prostaglandin E_2 reduces fibroblast growth. Each compound in micromolar concentration antagonizes serotonin, and at lower concentrations, norepinephrine and possibly histamine may potentiate the action of serotonin.

Materials and Methods. Fibroblasts[2] [mouse 3T6, L929, human embryo cells, and a strain developed in our laboratory from mouse embryo skin by the technique of Paul (2)], 4×10^4 cell/ml, were grown in 2 ml of Dulbecco's modification of Eagle's basal medium (Grand Island Biological Company) with 10% bovine calf serum and antibiotics (0.1 mg streptomycin and 100 U penicillin/ml) using a 2-oz glass prescription bottle as a culture flask. Cultures were flushed with a mixture of atmospheric air and carbon dioxide (90:10), sealed with rubber stoppers, and incubated at 34° in a light-tight incubator.

Most of the compounds were tested over a concentration range of 10^{-9}–10^{-5} *M* alone and in combination with serotonin (10^{-6} *M*). The compounds were included in the subculture medium and the cells grown for 72 hr. The response was compared with cultures containing tryptamine (10^{-6} *M*), previously shown to have no effect upon cell growth (1), and with cultures grown with serotonin (10^{-6} *M*). Histamine dihydrochloride, norepinephrine bitartrate, serotonin bimaleate, and tryptamine hydrochloride were used.

The cells were harvested after decanting the incubation medium, washing with chilled Hanks' solution, and then detaching the cells from the side of the culture flask with a 5-min incubation at 25° in 5 ml of a 0.6% trypsin:0.15% disodium EDTA solution. The cells were transferred to a centrifuge tube, sedimented at 900*g* for 10 min, and washed with Hanks' solution. The cell pellet was suspended in 0.15 *M* NaCl, and cell count was made on a portion of the suspension using a Coulter counter.

For studies with serially subcultured cells, a primary strain of mouse fibroblasts from embryo corium was sequentially cultured, and the effects of serotonin and histamine were measured after 2, 4, and 10 subcultures.

Results. Histamine and norepinephrine enhance fibroblast growth. The growth response to histamine occurred over a concentration range of 10^{-8}–10^{-5} *M*, with a maximum effect of 65% at 10^{-6} *M* (Fig. 1A). Nor-

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[2] 3T6 Fibroblasts were kindly supplied by Dr. Howard Green, N. Y. U.; L929 and human embryo lung fibroblasts were purchased from the American Type Culture Collection, Rockfield, MD.

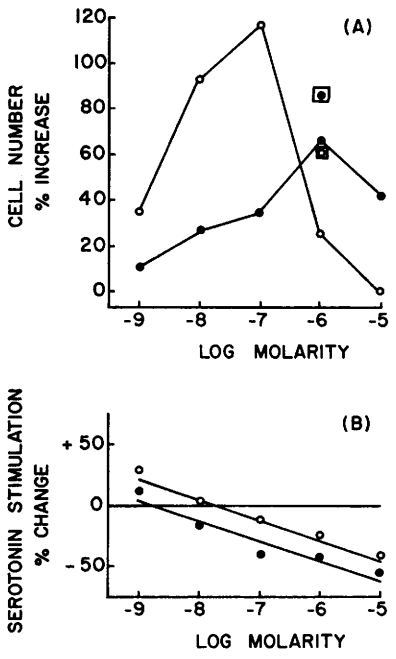

FIG. 1. Histamine, norepinephrine, serotonin, and fibroblast (3T6) growth. Values are the mean of at least three cultures. (A) Cells grown for 72 hr with different concentrations of histamine (●) or norepinephrine (○), and cell number related to values obtained for cells grown with tryptamine (10^{-6} *M*). Values for serotonin are indicated by (solid circle in box) in histamine and (open circle in box) in norepinephrine series. (B) Cultures grown 72 hr with serotonin (10^{-6} *M*) plus different concentrations of histamine (●) or norepinephrine (○), and values expressed as % change in stimulation obtained in cultures grown with serotonin (10^{-6} *M*) alone.

epinephrine enhanced growth over a concentration range of 10^{-9}–10^{-6} *M*, with a maximum effect of 118% at 10^{-7} *M*. By way of comparison, the growth-enhancement effect of 10^{-6} *M* serotonin determined at the same time was 87 and 61%, respectively. Previously, we observed that serotonin enhanced growth over a concentration range of 0.7–1.1 × 10^{-6} *M*, with a maximum at 10^{-6} *M* (3).

Histamine and norepinephrine were serotonin antagonists at concentrations of 10^{-6} and 10^{-5} *M* (Fig. 1B). At 10^{-9} *M*, norepinephrine potentiated the serotonin effect 34% ($p < 0.01$); the increase of 13% with histamine was not statistically significant.

Histamine, however, did not enhance the growth in all strains of fibroblasts. In fact, histamine inhibited the growth of human embryo lung fibroblasts and a primary culture of the strain from mouse embryo skin (Table I). In contrast, norepinephrine and serotonin enhanced growth in all fibroblast strains.

The variation in response to histamine that depends upon the strain of fibroblasts suggested that the histamine effect might be acquired perhaps by multiple subculturing, a possibility that was tested directly. A primary culture from mouse embryo skin was developed and serially cultured (Table II). After two subcultures, cell growth was not affected, but after 4 or 10 subcultures, histamine significantly enhanced growth by 20% ($p < 0.001$ in both subcultures). Serotonin, on the other hand, enhanced growth after each subculture.

Bradykinin at 10^{-6} *M* increased human fibroblast growth by 50%, but when combined at this concentration with serotonin, cell number did not differ from the control value. Bradykinin completely inhibited the growth response to serotonin. The specificity of the stereoconfiguration of the amino acids of the bradykinin molecule for fibroblast growth enhancement was shown by the inactivity of a bradykinin analog (D-pro-2, 3, 7). Prostaglandin E_2 at concentrations of 10^{-7} and 10^{-6} *M* inhibited growth of L929 and mouse embryo skin fibroblasts by 30%.

Discussion. These results indicate that all three biogenic amines, serotonin, histamine, and norepinephrine, can enhance fibroblast growth *in vitro*. The growth-enhancing effect with serotonin and norepinephrine occurs in all strains or lines of fibroblasts, suggesting that the response to these two amines is intrinsic to fibroblasts. In contrast, the growth response to histamine may be acquired, as suggested by studies with serial cultures of mouse fibroblasts (Table II).

Each biogenic amine has a distinctive dose–fibroblast growth response curve (Fig. 1A and Ref. 3), suggesting different mechanisms of action. One mechanism of action of these amines may be on the cellular levels of adenosine triphosphate and/or cyclic

TABLE I. Histamine, Norepinephrine, and Serotonin: Growth-Enhancing Effect on Different Fibroblast Strains.[a]

Cell source	Tryptamine	Histamine	Norepinephrine	Serotonin
	(Cell number × 10^{-5})			
Human embryo lung	10.7 ± 1.14[b] (6[c])	6.68 ± 2.82 (6)	14.2 ± 2.93 (6)	20.0 ± 2.50 (6)
Mouse embryo skin (primary cultures)				
No. 1	7.24 ± 0.47 (5)	4.11 ± 0.35 (5)	—	—
No. 2	5.36 ± 0.36 (4)	5.77 ± 0.28 (4)	6.28 ± 0.33 (4)	7.80 ± 0.37 (4)
Mouse fibroblast line (3T6)				
No. 1	14.2 ± 1.10 (3)	—	17.8 ± 1.10 (3)	22.9 ± 1.85 (3)
No. 2	13.2 ± 1.30 (4)	21.8 ± 6.22 (4)	—	24.7 ± 4.45 (4)

[a] Cultures of fibroblasts grown 72 hr with histamine, norepinephrine, or serotonin and compared with cultures grown with tryptamine. Each amine at 10^{-6} *M* concentration.
[b] Mean ± SD.
[c] Number of cultures.

nucleotide monophosphates. Serotonin has been associated with ATP formation in a variety of cells, and it is reported to stimulate oxidative phosphorylation of rat mitochondria (4).

Considerable indirect information is appearing relating fibroblast growth rate and endogenous levels of the cyclic nucleotide monophosphates. The cellular level of cyclic adenosine 3′,5′-monophosphate (cyclic AMP) influences fibroblast morphology, and cell division, during the logarithmic growth period, is inversely correlated with the endogenous level of cyclic AMP (5). Fibroblasts cultured with the dibutyryl derivative of cyclic AMP grow more slowly than untreated cells (6). Mouse fibroblasts (L929, 3T3, and 3T6) in the presence of prostaglandin E_1 have an activated adenyl cyclase (7) and elevated endogenous levels of cyclic AMP (8), and growth rate is decreased (9). Prostaglandin E_2 has a similar, though slightly less effective, action (7). In our experiments, prostaglandin E_2 inhibited fibroblast growth.

TABLE II. Growth-Enhancing Effect of Histamine and Serotonin on Serially Subcultured Fibroblasts.[a]

Number of cell transfers	Tryptamine	Histamine	Serotonin
	(Cell number × 10^{-5})		
2	5.36 ± 0.36[b] (4[c])	5.77 ± 0.28 (4)	7.80 ± 0.37 (4)
4	5.29 ± 0.21 (5)	6.21 ± 0.27 (5)	7.00 ± 0.21 (4)
10	5.74 ± 0.40 (5)	6.92 ± 0.27 (5)	7.26 ± 0.28 (5)

[a] Mouse embryo skin fibroblasts serially subcultured and then grown 72 hr with histamine or serotonin and compared with cultures grown with tryptamine. Each amine at 10^{-6} *M* concentration.
[b] Mean ± SD.
[c] Number of cultures.

Makman's findings (10) that serotonin over a wide concentration range did not activate adenyl cyclase of fibroblasts (mouse 3T6) do not exclude the possibility that serotonin may act via the cyclic nucleotide mechanism, *e.g.*, cyclic guanosine 3′,5′-monophosphate (cyclic GMP). Hormones may act via the cyclic AMP mechanism while not affecting cyclic GMP levels (11). Cyclic adenosine 3′,5′-monophosphate and its dibutyryl derivative inhibit [H^3]thymidine incorporation while

cyclic GMP does not inhibt [H^3]thymidine uptake (12). However, a suggestion that serotonin acts via the cyclic nucleotide mechanism is purely speculative at this time.

The histamine effect on fibroblasts is interesting in this regard. Histamine enhances fibroblast growth only in the 3T6 line and in cells that have undergone multiple subculturing. Makman (10) reports an active adenyl cyclase system in mouse embryo fibroblasts (3T3 and 3T6 lines), both of these cell lines being products of multiple subculturing, while "several primary cultures of human fibroblasts had very low adenyl cyclase activity". Histamine is known to accelerate adenyl cyclase activity in a variety of tissues (13, 14), and perhaps the action of histamine is mediated by acquired changes in the adenyl cyclase activity in fibroblasts.

Norepinephrine has a broad concentration range over which fibroblast growth enhancement occurs and, like serotonin, enhances growth of all fibroblasts studied. Makman (10) reports a norepinephrine-sensitive adenyl cyclase in mouse 3T6 and 3T3 fibroblasts and a norepinephrine-insensitive enzyme in human fibroblasts. In human subjects, it has been shown that the β-adrenergic effect of norepinephrine is mediated via cyclic AMP while its α-adrenergic effect is via cyclic GMP (15). It may be that the fibroblast growth response to norepinephrine can be mediated by either the adenyl or guanyl cyclase system.

In the 3T6 fibroblast line, histamine and norepinephrine may act to either antagonize or potentiate the effect of serotonin, depending on their concentration. The antagonistic effect at high concentrations could be due to an interference with serotonin uptake or an interaction with a receptor site, but this would not explain the potentiation effect of norepinephrine. A more likely possibility is that a varying concentration of product(s) of histamine or norepinephrine stimulation either antagonizes or enhances the serotonin effect.

Bradykinin enhances and prostaglandin E_2 reduces cell growth while the D-pro-2, 3, 7 stereoisomer of bradykinin is inactive. This observation suggests that the fibroblast "recognizes" the configuration of the biologically active form of bradykinin just as Spragg *et al.* (16) report for the spatial specificity of the interaction between bradykinin and its antibody.

The findings that the biogenic amines and bradykinin enhance fibroblast growth open the possibility that compounds, heretofore identified as chemical regulators of the vascular response to tissue injury, may also modulate the repair process by directly acting upon fibroblast growth and metabolism.

Summary. Histamine and norepinephrine have a concentration-dependent growth-enhancing effect on fibroblast growth *in vitro* that is different from the response to serotonin. These distinct dose–response curves suggest different mechanisms of action. Furthermore, histamine depresses growth of human embryo lung fibroblasts while enhancing growth of an established cell line (3T6) and serially cultured strains of mouse skin fibroblasts. The acquired nature of the histamine response is in contrast to the consistent growth-enhancing effect of serotonin and norepinephrine on all fibroblast lines or strains.

At micromolar concentrations, histamine, norepinephrine, and bradykinin antagonize the action of serotonin, while at a nanomolar level norepinephrine potentiates the effect of serotonin. The spatial configuration of bradykinin is critical for the fibroblast growth response. Prostaglandin E_2 inhibits fibroblast growth.

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