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Effects of Bradykinin on Rat Lymphocytes (39557)

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Vasoactive peptides (bradykinin and related kinins) are released in injured tissues from protein substrates by a variety of proteolytic enzymes. Kinins are considered to mediate inflammatory processes due to their ability to induce vasodilation, increased blood vessel permeability, and pain. Recently to the list of these well-recognized physiological effects of bradykinin, a new one has been added, namely, stimulation of mitotic activity of rat thymocytes, possibly by a cAMP-mediated reaction (1, 2). This, if confirmed, for peripheral lymphatic cells would enlarge considerably the role of bradykinin in mediation of tissue response to injury, since the stimulated lymphatic cell may be a source of multiplicity of factors affecting the inflammatory reaction and wound healing.

The aim of the present work is to investigate the effects of bradykinin on lymphocytes from rat peripheral lymph nodes.

Materials and methods. Female Wistar rats, 3- to 4-months old, were bled under ether anesthesia, and then axillary, submandibular, inguinal, and mesenteric lymph nodes were removed. Lymphocyte suspensions were prepared by dissection of lymph nodes with preparative needles and placed in the medium 199 (Biomed, Lublin, Poland). Bradykinin (Koch Light Lab., Colnbrook, England) was dissolved in the medium 199. [^{3}H]Thymidine, 2 Ci/mmole, was purchased from UVVVR, Prague, Czechoslovakia. [^{3}H]uridine, 20–30 Ci/mmole, [^{14}C]leucine, 62 mCi/mmole, and NCS tissue solubilizer were the products of Radiobiological Centre, Amersham, England. Lymphocytes (10^7) were cultivated in medium 199, supplemented with 20% (vol/vol) of calf serum and 0.03% L-glutamine, in final volume samples of 2 ml, at 37° for various times up to 96 hr. Bradykinin was added at the beginning of cultivation.

Incorporation of [^{3}H]thymidine and [^{3}H]uridine was determined as described by Bauscher *et al.* (3). Washing of TCA precipitates with methanol was omitted. [^{3}H]-Thymidine (2 μCi per culture) or [^{3}H]-uridine (1 μCi per culture) was added 18 or 24 hr before the cell harvest, respectively.

Incorporation of [^{14}C]leucine (1 μCi per culture) added 4 hr before cell harvest was determined as described by Kay *et al.* (4). TCA precipitates were separated by centrifugation at 1000 *g* for 15 min (instead of separation on Millipore filters) and dissolved in 0.5 ml of NCS tissue solubilizer. Radioactivity measurements were carried out using POP-POPOP-toluene liquid scintillator and Mark II Nuclear Chicago counter.

Viability of lymphocytes was estimated by 0.2% trypan blue exclusion test. Bradykinin concentrations used did not affect significantly cell numbers and viability during the 96-hr cultivation, as compared with the appropriate controls.

Results. Effects of bradykinin on incorporation of [^{3}H]thymidine, [^{3}H]uridine, and [^{14}C]leucine are summarized in Tables I, II, and III, respectively. The data represent mean values from 10 to 15 determinations with the standard deviations. Incorporation of the precursors was markedly enhanced by all concentrations of bradykinin employed. At its lowest level, 1.0 μM, the increments over the control values were moderate (16–43%) but significant ($P < 0.05$) after 24 hr of culture, and they increased rapidly to 200–300% of control values after 72 hr. No regular dependence of the incorporation on bradykinin concentration was observed. However, the values found at 10- μM bradykinin concentration tend to exceed those found at 1 and 5 μM of bradykinin.

The stimulating activity of all applied bradykinin concentrations was evident as early as after 24 hr. The maximal increase in

TABLE I. EFFECT OF BRADYKININ ON [^{3}H]THYMIDINE INCORPORATION INTO LYMPHOCYTES (CPM × 10^{-3} PER SAMPLE).

Bradykinin (μM)	Cultivation time (hr)			
	24	48	72	96
	(cpm × 10^{-3} per sample)			
0	3.2 ± 0.4	2.6 ± 0.3	1.6 ± 0.4	1.4 ± 0.2
1.0	4.2 ± 0.2	4.8 ± 0.3	5.4 ± 0.9	3.3 ± 0.2
5.0	4.0 ± 0.2	4.6 ± 0.4	5.0 ± 0.3	3.1 ± 0.4
10.0	4.5 ± 0.2	5.7 ± 0.7	8.0 ± 1.0	5.1 ± 0.9
20.0	4.2 ± 0.3	4.9 ± 0.4	5.3 ± 0.6	2.4 ± 0.5

TABLE II. EFFECTS OF BRADYKININ ON SPONTANEOUS INCORPORATION OF [^{3}H]URIDINE INTO LYMPHOCYTES (CPM × 10^{-3} PER SAMPLE).

Bradykinin (μM)	Cultivation time (hr)			
	24	48	72	96
	(cpm × 10^{-3} per sample)			
0	56.3 ± 3.3	29.1 ± 2.9	23.2 ± 3.3	18.8 ± 4.5
1.0	65.2 ± 2.8	68.5 ± 5.5	53.3 ± 6.8	49.7 ± 7.0
5.0	72.1 ± 2.5	79.7 ± 6.8	51.1 ± 3.5	52.6 ± 4.1
10.0	89.3 ± 4.9	101.7 ± 9.3	74.3 ± 7.3	62.3 ± 5.0
20.0	89.7 ± 3.8	87.8 ± 7.1	56.6 ± 3.2	44.5 ± 2.7

TABLE III. EFFECTS OF BRADYKININ ON SPONTANEOUS INCORPORATION OF [^{14}C]LEUCINE INTO LYMPHOCYTES (CPM + 10^{-3} PER SAMPLE).

Bradykinin (μM)	Cultivation time (hr)			
	24	48	72	96
	(cpm × 10^{-3} per sample)			
0	1.6 ± 0.1	0.8 ± 0.3	0.7 ± 0.3	0.6 ± 0.3
1.0	2.3 ± 0.2	2.5 ± 0.4	2.1 ± 0.4	1.5 ± 0.3
5.0	2.1 ± 0.2	2.4 ± 0.4	1.9 ± 0.2	1.6 ± 0.3
10.0	2.4 ± 0.3	3.0 ± 0.6	2.1 ± 0.2	1.6 ± 0.2
20.0	2.5 ± 0.3	2.7 ± 0.5	2.3 ± 0.3	1.8 ± 0.3

[^{3}H]thymidine incorporation was observed after 72 hr while maximal effects of bradykinin on incorporation of [^{3}H]uridine and [^{14}C]leucine were detected 24 hr earlier, i.e., after 48-hr cultivation.

Discussion. A number of observations reported recently indicates that low-molecular regulatory substances, such as catecholamines, prostaglandins, histamine (5), as well as peptide hormones, influence the proliferation and function of lymphatic cells. Some of the peptide hormones, such as growth hormone (6), parathormone (7), and vasopressin (8), were investigated mainly in respect to their stimulatory effect on proliferation of thymocytes.

On the contrary, chorionic gonadotropin and chorionic somatomammotropin were found to inhibit the blastogenic response of human lymphocyte. These effects of placental hormones were postulated to be of a probable significance for maternal lymphocyte immunocompetence and fetus survival (9–12).

Information about the effects of bradykinin and related kinins on lymphatic tissue is scarce. Perris and Whitfield (1) and Whitfield *et al.* (2) showed that bradykinin stimulates incorporation of [^{3}H]thymidine and mitotic activity in rat thymocytes *in vitro*. The data reported in this paper reveal a strong effect of bradykinin on peripheral lymph node cells of rat. Similarly to the above-mentioned authors, we have observed a pronounced increase of [^{3}H]thymidine incorporation, and have found that it was accompanied by an increased incorporation of [^{3}H]uridine and [^{14}C]leucine. As these effects did not seem to be concentration dependent, the lowest brady-

kinin concentration employed, 1.0 μM, is perhaps sufficient to generate the maximum response resulting in a plateau at all other concentrations. However, shape of the dose-response curve could have been affected by the presence of serum in the culture medium, since presumably it contained active kininases. The addition of serum was, of course, necessary to maintain viability of the lymphocytes during the 96-hr cultivation. This would tend to equalize responses at various concentration levels, the notion supported by a rather consistent occurrence of higher precursor incorporation at a high (10 μM) bradykinin concentration. The stimulating effect of bradykinin below 1.0 μM has not been as yet, to our best knowledge, investigated in lymphatic cells. It now becomes important to estimate the lowest effective bradykinin concentration in order to assess the relevance of the observed phenomena for physiological processes.

The time course of the incorporation differed with various precursors tested. Peak values for [^{3}H]uridine and [^{14}C]leucine incorporation occurred after 48-hr incubation and were followed by [^{3}H]thymidine maximum at 72 hr. This sequence of events is considered to characterize the response of lymphocytes to specific antigens, as well as to nonspecific mitogens (13). As the lymphocytes used in the present studies were cultivated in the presence of heterologous calf serum, the question arises whether the bradykinin-enhanced stimulation of protein and nucleic acid synthesis is connected with, or is independent of activation of, the lymphocyte immumological functions. It seems likely that the effects observed in this work are caused by the direct action of bradykinin, since Perris and Whitfield (1) and Whitfield *et al.* (2) have shown that the mitotic activity of thymocytes was stimulated by bradykinin in a serum-free medium.

Summary. Bradykinin stimulates incorporation of [^{14}C]leucine, [^{3}H]uridine, and [^{3}H]thymidine into cultured lymphocytes of rat lymph nodes. Increase in [^{14}C]leucine and [^{3}H]uridine incorporation precedes that of [^{3}H]thymidine.

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