The in vitro Effect of Adrenergic Agents and Related Compounds on Triglyceride Levels of Guinea-Pig Lymphoid Cells

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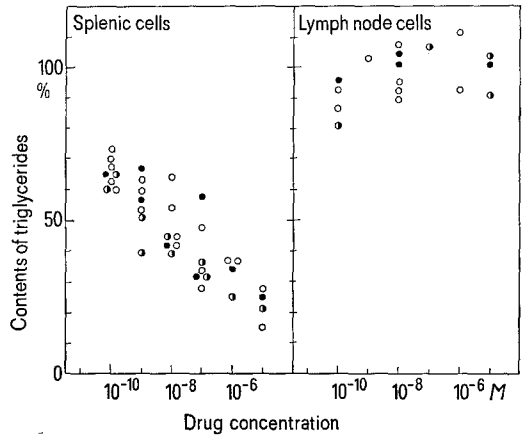
Summary. Isoproterenol, epinephrine and norepinephrine were highly potent in decreasing the triglyceride levels of the splenic lymphoid cells from guinea-pigs in vitro, while they were quite inert upon the inguinal lymph node cells.

There is increasing evidence that several functions of the immune system are intimately associated with the adrenergic receptors in the plasma membrane of lymphocyte[1–6]. In a previous work we reported the characteristic distribution patterns in the lipid constituents of guinea-pig lymphoid cells[7]. This raises the question as to whether the adrenergic drugs, known to stimulate lipolytic activity in adipose tissues[8], might influence the lipid contents of lymphoid cells. The present study demonstrates that the triglyceride levels of the splenic lymphoid cells from guinea-pigs are highly sensitive to adrenergic catecholamines.

Guinea-pigs of Hartley strain, weighing 500–700 g, were injected with Freund's complete adjuvant on footpad. 2 weeks after injection, the spleen and inguinal lymph node were removed from the animals, which had been fed with diet and given water ad libitum. Suspensions of lymphoid cells from spleen and lymph node were prepared following the procedure described previously[7]. The medium used was Krebs-Ringer phosphate buffer, pH 7.4 (KRP)[9]. For each experiment, spleens and lymph nodes from at least 4 or 8 animals, respectively, were pooled, yielding approximately 0.4 g (wet weight) of lymphoid cells. The washed cells were finally suspended in an appropriate volume of KRP containing 2% of bovine albumin fraction V (Armour Laboratories), supplemented with 100 IU/ml penicillin and 100 µg/ml streptomycin, so as to contain approximately 5×10^6 cells/ml with a viability greater than 90% by trypan blue test. The cell suspensions were distributed to 200-ml flasks in about 50 ml aliquots, and the flasks incubated at 37 °C for 2 h with shaking in an atmosphere of air. At the end of the incubation, the cells were harvested by washing in KRP. The triglycerides of the washed cells were extracted and analyzed as reported elsewhere[7]. In assessment of the effect of catecholamines on the lipid levels, the values determined from the cells incubated in the presence of drugs were evaluated as percentage of the drug-free controls. The compounds used were obtained commercially.



Effect of catecholamines on the triglyceride contents of lymphoid cells from spleen and inguinal lymph node of guinea-pigs. Symbols: ○, isoproterenol; ◑, epinephrine; ●, norepinephrine.

The log dose-percent response relationships obtained from 3 adrenergic agonists, isoproterenol, epinephrine and norephinephrine, are presented in the Figure. Under the experimental conditions used in this study, the response to drugs of the lymphoid cells from the pooled tissues varied greatly from day to day. However, a comparison of these dose-response figures reveals that the 3 agonists were capable of producing a similar, but unique for each cell source, response of the triglyceride levels of lymphoid cells. Thus, the splenic cells were extraordinarily sensitive to the catecholamines: When the splenic cells were incubated for 2 h in 10^{-5} M concentration of the drugs, the triglyceride levels fell to about 25% of the original levels (triglycerides/dried cells: 28.9 $\pm$ 6.5 mg/g for control cells and 6.6 $\pm$ 1.9 mg/g for drug-treated cells, $p < 0.05$). The response to logarithmically decreased dose of the 3 drugs decreased in a linear fashion (correlation coefficient $r = 0.88$ and regression coefficient $b = -0.83$, $p < 0.01$) over the range from 10^{-5} to 10^{-10} M, and approached the control lipid levels at a concentration of 10^{-10} M (triglyceride contents: 22.6 $\pm$ 3.5 mg/g for control cells and 14.7 $\pm$ 2.3 mg/g for drug-treated cells, $p < 0.1$). Their 50% decreasing dose was approximately 10^{-8} M. Surprizingly, at lower concentrations beyond 10^{-10} M the drugs caused sometimes a significant decrease far over the range of daily variations in the triglyceride contents of the splenic cells (triglyceride contents at concentrations of 10^{-11} to 10^{-20} M, $n = 12$: 36.7 $\pm$ 7.3 mg/g for control cells and 27.0 $\pm$ 6.2 mg/g for drug-treated cells, $0.4 > p > 0.2$). The reason of these inconsistent and apparently paradoxical variations in the drug responsiveness, observed in the range of picomolar or less concentrations, is unknown at the present time. The effect of phenylephrine was considerably less than that observed with the three catecholamines, the 50% decreasing dose being approximately 10^{-6} M, while ephedrine had practically no effect over the range of concentrations 10^{-10}–10^{-3} M (percent contents of triglycerides: 43–85% for phenylephrine and 71–107% for ephedrine). These results were very similar to those

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Effect of adrenergic blocking agents on triglyceride content of splenic cells when added alone or with adrenergic catecholamines[a]

Experiment number		Drugs	Concentration (M)	Content of triglycerides (%)
I	1	Isoproterenol	10^{-8}	64.4
	2	Propranolol	10^{-7}	51.1
	3	Isoproterenol + Propranolol	10^{-8} + 10^{-7}	21.5
II	1	Epinephrine	10^{-9}	40.4
	2	Phentolamine	10^{-6}	12.4
	3	Epinephrine + Phentolamine	10^{-9} + 10^{-6}	11.3

[a]Adrenergic catecholamines were added 5 min after the addition of the blockers.

reported by FELLER and FINGER[10] in rat epididymal fat tissue. In marked contrast, under similar conditions none of the adrenergic agents active upon splenic cells caused any significant change in the triglyceride levels of the lymph node cells, even when incubated in concentrations as high as 10^{-5} M (triglyceride contents: 39.6 $\pm$ 3.6 mg/g for control cells and 39.0 $\pm$ 3.5 mg/g for drug-treated cells, $p > 0.8$) (Figure). Whether or not this contradiction between the two lymphoid cell populations of different sources merely reflects the vast complexities of each pooled cell population[11–15], remains unclear. The time course of the effects of isoproterenol on splenic cells was followed for ranging up to 6 h. Although the time course considerably varied with concentrations, a measurable decrease in triglyceride contents was produced within 1/2 to 1 h, reaching the maximal response after 2 to 4 h. On 6-hour incubation, the lipid levels rose toward the control levels. In an attempt to determine the mode of action for adrenergic agents, the effects of the catecholamines were evaluated in the presence of α- and β-adrenergic blocking agents. In the Table are given the results of 2 experiments in which isoproterenol, epinephrine, propranolol and phentolamine were added as indicated. Unexpectedly, both propanolol and phentolamine, when added singly in the splenic cell suspensions, caused a dramatic lowering of the lipid levels, and the isoproterenol- or epinephrine-induced reduction of the lipid contents were unaffected by the presence of either of the adrenergic blockers. Interestingly, fatty acids have recently been shown to be involved in corticosteroid-induced lymphocytolysis (TURNELL et al.[16]) and in the regulatory mechanism for cell-mediated immunity by prostaglandins (MERTIN and HUGHES[17]). The present results suggest an involvement of lipid metabolism in physiological functioning of lymphocytes through adrenergic receptor system.

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Effect of ACTH on the Zona Reticularis of the Rat Adrenal Cortex: an Ultrastructural Stereologic Study

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Summary. The effects of ACTH on the rat adrenal *zona reticularis* were investigated by stereologic methods. It was found that the *zona reticularis* cell responsiveness to ACTH is similar to that of the *zona fasciculata* elements. This excludes that the *zona reticularis* of the adult rat can only function as the site of destruction of worn-out elements migrating from the adrenal outer zones.

Although the ultrastructure of the adrenal cortex in normal and experimental conditions was described in numerous investigations[2], until recently very scarce reports appeared concerning the cytophysiology of the adrenal zona reticularis[3]. However, the study of the zona reticularis is of great interest, since at present the role played by this zone in the histophysiology of the entire adrenal gland is still the object of conflicting views, which vary according to the 'zonal hypothesis' or the 'migration theory'[2]. It therefore seemed worth investigating, by stereologic and electron microscopic methods, the response of the rat adrenal zona reticularis to a chronic treatment with ACTH.

Materials and methods. 42 young male rats (Wistar-derived) weighing about 200 g, were divided into 7 experimental groups, of which 6 received i.p. injections of 10 IU/kg of ACTH (Sigma) for 3, 6, 9, 12, 24 and 36 consecutive days, respectively. The other group received i.p. injections of normal saline and served as a control. The animals were sacrificed by cervical dislocation.

The right adrenal of each rat was fixed in 10% buffered formalin, embedded in paraffin and serially cut at 7 µm. Sliced pieces of the left gland were fixed in 3% glutaraldehyde, post-fixed in 1% OsO_4 and embedded in an epoxy resin. Thick sections were made with LKB III ultramicrotomes and observed with the light microscope for orientation. Thin sections were cut at the level of the zona reticularis and observed in a Hitachi HU-12 or HS-9 electron microscope.

On the serial paraffin sections, the volume of the entire adrenal gland and of the zona reticularis was determined using a method previously detailed[4]. The volume of cells,