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Biochemical Pharmacology, Vol. 29, pp. 3330–3333.

0006-2952/80/1215-3330 $02.00/0

Alpha$_1$-adrenergic activation of phosphatidylinositol labeling in isolated brown fat cells*

(*Received* 14 *May* 1980; *accepted* 7 *July* 1980)

In the early 1960's, Smith and Hock [1] suggested that the primary physiological function of brown adipose tissue is that of heat production. It is now considered that brown adipose tissue is the primary thermogenic effector organ in arousing hibernators, most cold-exposed newborns and cold-exposed adults of many non-hibernating species [2]. Brown adipose tissue is richly innervated, and catecholamines seem to play a major physiological role in modulating its activity.

Electrophysiological studies have demonstrated depolarization of brown adipocytes in response to endogenous (nerve stimulation) or exogenous catecholamines [3–5]. Recently, Fink and Williams [6] have shown that both alpha and beta adrenoceptors mediate depolarization in brown adipocytes.

Alpha adrenoceptors have been subdivided in two subtypes—alpha$_1$ and alpha$_2$ [7, 8]. Fain and Garciá-Sáinz [9] have suggested that the subdivision of the alpha adrenoceptors is both strucural (affinity for agonists and antagonists) and functional (underlying mechanism of action). Activation of alpha$_1$ adrenoceptors mediates those effects of catecholamines that involve phosphatidylinositol turnover and the entry or mobilization of calcium, whereas activation of alpha$_2$ adrenoceptors mediates those effects of adrenergic amines resulting from inhibition of adenylate cyclase [9]. The present experiments were designed to determine whether an alpha-adrenergic stimulation of phosphatidylinositol turnover can be seen in brown fat cells.

Epinephrine, isoproterenol, and propranolol were obtained from the Sigma Chemical Co. (St. Louis, MO) yohimbine from ICN Nutritional Biochemicals (Cleveland, OH) crude collagenase (*Clostridium histolyticum*) from the Worthington Biochemical Corp. (Freehold, NJ) (Lot No. CLS 48A281), bovine serum albumin (Fraction V) from the Armour Pharmaceutical Co. (Kankakee, IL) (Lot No. S 11709) and [^{32}P]P$_i$ as orthophosphoric acid (carrier-free) from the New England Nuclear Corp. (Boston, MA). Prazosin and phentolamine were provided by Pfizer Inc. (Groton, CT) and the CIBA Pharmaceutical Co. (Summit, NJ) respectively.

Brown fat cells were isolated from the dorsal interscapular brown adipose tissue of Sprague–Dawley rats (Charles River CD strain) according to the method of Fain *et al.* [10] with minor modifications. In brief, brown adipose tissue from ten to fifteen rats was removed and carefully trimmed of adhering skeletal muscle or white adipose tissue; the tissue was digested in buffer containing 0.75 mg collagenase/ml. After 20 min, the non-digested tissue was filtered onto a layer of nylon chiffon, and the remaining pieces of brown adipose tissue were cut into small pieces and incubated for 40 min in buffer containing 1.5 mg collagenase/ml. This procedure is similar to that of Pettersson and Vallin [11] and allowed us to get at least 80% multilocular fat cells.

The incorporation of [^{32}P]P$_i$ into phospholipids was studied as described previously for white fat cells [12] with some modifications. In brief, brown fat cells (about 10^5 cells) were incubated in 1 ml of buffer containing 6% albumin and 10 μCi/ml of [^{32}P]P$_i$ for 60 min in a water bath shaker at 37°. Lipids were extracted with chloroform–methanol (2:1), and phospholipids separated by one-dimensional thin-layer chromatography as described previously [12]. The phosphorus content of each phospholipid was determined by the microprocedure of Bartlett [13]. Krebs-Ringer Tris buffer of the following composition was used in all the experiments: 120 mM NaCl, 1.4 mM $CaCl_2$, 5.2 mM KCl, 1.4 mM $MgSO_4$ and 5 mM Tris. The buffer was prepared daily and adjusted to pH 7.4 at 37° with NaOH after addition of the albumin powder.

Cyclic AMP accumulation was measured at 10 min in the presence of adenosine deaminase (0.5 μg/ml) and theophylline (100 μM) by a modification of the method of Gilman [14].

Incubation of brown cells in buffer containing radioactive phosphate resulted in significant incorporation of label into phospholipids. The specific activity of major phospholipids was as follows: cardiolipin + phosphatidylglycerol 135 ± 45 cpm/μg phosphate; phosphatidylethanolamine, 150 ± 15 cpm/μg phosphate; phosphatidylcholine, 865 ± 150 cpm/μg phosphate; phosphatidylinositol, 1195 ± 305 cpm/μg phosphate; and phosphatidic acid + phosphatidylserine, 819 ± 150 cpm/μg phosphate. The values are the means ± S.E.M. of seven experiments.

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† International fellow sponsored by the N.I.H. (Fellowship IFO 5TWO 2691).

Table 1. Effect of propranolol, epinephrine, and isoproterenol on the labeling of brown fat cell phospholipids*

Addition	Per cent of control specific activities				
	CL + PG	PE	PC	PI	PA + PS
(±)-Propranolol (30 μM)	125 ± 10	150 ± 20	95 ± 10	175 ± 20	125 ± 10
(−)-Epinephrine (10 μM)	85 ± 5	85 ± 10	65 ± 5	205 ± 25	135 ± 10
(−)-Epinephrine (10 μM)+ (±)-propranolol (30 μM)	125 ± 15	150 ± 15	75 ± 5	535 ± 110	185 ± 20
(−)-Isoproterenol (10 μM)	92 ± 10	70 ± 10	85 ± 10	65 ± 10	115 ± 15

* Values are the means ± S.E.M. of seven experiments performed on different days. The control specific activity of each phospholipid is given in the text. Abbreviations: CL, cardiolipin; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; PA, phosphatidic acid; and PS, phosphatidylserine.

Addition of epinephrine to brown fat cells resulted in a dose-dependent increase in the incorporation of radioactive phosphate into phosphatidylinositol and its precursor phosphatidic acid concomitant with a decrease in the specific activities of the other phospholipids (Fig. 1). Propranolol also slightly increased phosphatidylinositol specific radioactivity without affecting the labeling of other phospholipids (Table 1). This effect of propranolol has been observed in other systems [12, 15–17] and seems to be due to its local anesthetic properties. These drugs exert their effect on phospholipid metabolism by redirection of synthesis toward phosphatidylinositol, probably by inhibiting phosphatidate phosphohydrolase [15–17].

The action of epinephrine on the labeling of phosphatidylinositol and phosphatidic acid plus phosphatidylserine was magnified by propranolol (Table 1). The pure beta-adrenergic agent isoproterenol decreased the incorporation of phosphate into phospholipids, especially phosphatidylinositol (Table 1). The decrease in phospholipid labeling observed with beta-adrenergic agonists seems to be related to intracellular accumulation of free fatty acids [12]. A role of other factors, such as cyclic AMP, however, cannot be ruled out.

Alpha-adrenergic antagonists inhibited the effect of epinephrine on phosphatidylinositol labeling (Fig. 2). The potency order was: prazosin > phentolamine >> yohimbine, indicating that the alpha receptor involved in this action was $alpha_1$.

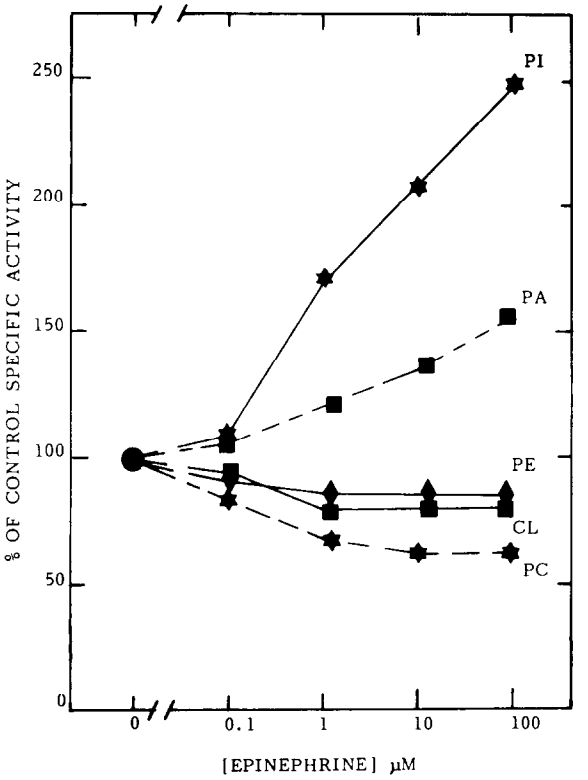

Fig. 1. Effect of epinephrine on the incorporation of $[^{32}P]P_i$ into brown fat cell phospholipids. The values plotted are the means of seven experiments performed on different days. The standard errors of the data are about 15 per cent of the values. The control specific activity of each phospholipid is given in the text. Abbreviations: PI, phosphatidylinositol (✱—✱); PA, phosphatidic acid (■--■); PE, phosphatidylethanolamine (◆—◆); CL, cardiolipin (■—■); and PC, phosphatidylcholine (✱--✱).

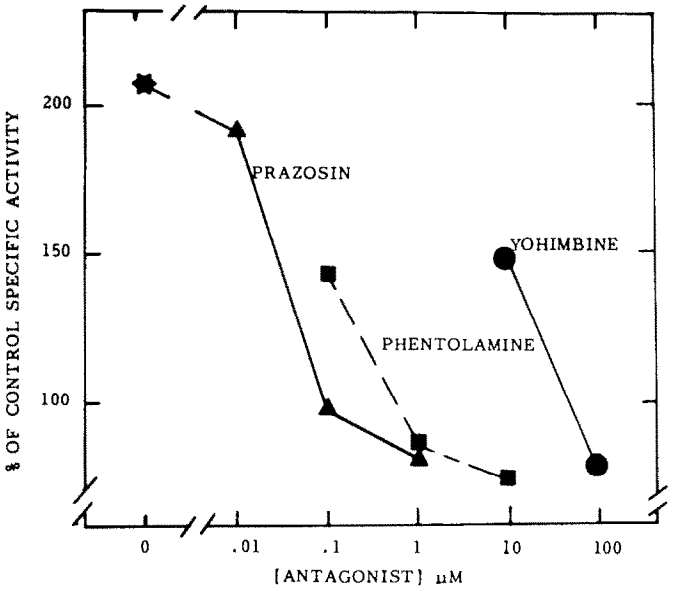

Fig. 2. Effect of alpha-adrenergic antagonists on the labeling of phosphatidylinositol in known fat cells produced by epinephrine. Brown fat cells were incubated with 10 μM epinephrine alone [✱] and with various concentrations of prazosin (▲—▲), phentolamine (■--■), and yohimbine (●—●). Other indications as in Fig. 1.

Cyclic AMP accumulation in response to 10 μM epinephrine was not significantly affected by alpha-adrenergic antagonists (data not shown). Cyclic AMP accumulation induced by 10 μM isoproterenol was also unaffected by the alpha$_2$-adrenergic agonist clonidine (0.1 to 10 μM) (data not shown).

An enhanced incorporation of radioactive phosphate into phosphatidylinositol and its precursor phosphatidic acid has been observed in response to hormones that increase the concentration of ionic calcium in the cytoplasm [18, 19]. Recently, Salmon and Honeyman [20] and Putney *et al.* [21] have suggested that phosphatidic acid is a calcium ionophore. At present, however, there is no clear-cut information about the link between receptor activation, increased turnover of phosphatidic acid plus phosphatidylinositol, and calcium mobilization.

We have postulated that activation of phosphatidylinositol metabolism and calcium mobilization by adrenergic amines is due to stimulation of alpha$_1$ adrenoceptors [9]. This has been shown in the pineal gland [22], rat and hamster white adipocytes [12, 23], rat hepatocytes [24], and now in brown adipocytes. Stimulation of alpha$_1$ adrenoceptors in hepatocytes produces an activation of glycogenolysis and glyconeogenesis [24–27]. In adipocytes, alpha$_1$–adrenergic stimulation results in an inactivation of glycogen synthase [28]. The physiological role of alpha$_1$ adrenoceptors in brown fat cells is unknown at present. The decrease in membrane potential produced by alpha-adrenergic stimulation in brown adipocytes is accompanied by an increase in membrane conductance [29]. Putney [30] suggested, as a general scheme for receptor control of permeability, that (a) occupation of receptors triggers a breakdown of phosphatidylinositol, which activates membrane calcium channels; (b) calcium moves into the cell passively down its electrochemical gradient, which activates Na^+ and K^+ channels; and (c) the passive fluxes of K^+ and Na^+ down their respective electrochemical gradient leads to a decrease in intracellular K^+ and in increase in Na^+, which activates the Na^+, K^+ pump. Consistent with this scheme, Girardier and Seydoux [31] found an influx of Na^+ and an efflux of K^+ after the addition of catecholamines to brown adipocytes. Horwitz [32] has suggested that the active transport of sodium and potassium contributes significantly to catecholamine-mediated brown fat cell thermogenesis. It has been suggested, however, that the decrease in catecholamine-activated respiration seen when the active transport of sodium and potassium is blocked by ouabain is secondary to a decrease in intracellular K^+, which is required for activation of thermogenesis [33, 34]. The activation of respiration is due to an uncoupling of mitochondrial oxidative phosphorylation by fatty acids released during lipolysis [33, 34].

Mohell *et al.* [35] have attempted to differentiate between alpha- and beta-adrenergic respiratory responses in hamster brown fat cells. They concluded that alpha-adrenergic contribution to thermogenesis could, at most, be responsible for no more than 10–20 per cent of norepinephrine action based on the ability of phentolamine to inhibit norepinephrine- or epinephrine-activated respiration [35]. Phentolamine, however, also inhibited the increase in respiration due to isoproterenol. What is needed are studies with prazosin, which is a selective alpha$_1$-adrenergic antagonist effective at very low concentrations.

Alpha-adrenergic agents decrease cyclic AMP accumulation in human [36] and hamster adipocytes [23, 37]. We have shown that this effect is mediated through activation of alpha$_2$ adrenoceptors and suggested that it is due to inhibition of adenylate cyclase [9, 23]. No evidence of alpha$_2$-adrenergic modulation of cyclic AMP accumulation was obtained in the rat white adipocytes [23]. Itaya [38] recently reported that phentolamine can further stimulate lipolysis enhanced by adrenaline or noradrenaline and postulated the existence of alpha adrenoceptors with inhibitory action on lipolysis in rat brown fat cells. We have been unable to show any clear cut action of alpha-adrenergic agents on the accumulation of cyclic AMP in brown fat cells. Phentolamine at high concentrations has many nonspecific actions and interference with epinephrine action does not necessarily result from a specific effect on alpha adrenoceptors [39, 40]. Our results do not rule out the presence of alpha$_2$ adrenoceptors in rat brown fat cells but suggest that, if present, they play a minor role in the regulation of cyclic AMP levels, compared with those in human or hamster adipocytes.

Alpha adrenoceptors have been demonstrated in intact brown adipocytes and crude homogenates of brown fat

from hamsters by Svartengren *et al.* [41] using [^{3}H]dihydroergocryptine. This ligand binds with equal affinity to both alpha$_1$ and alpha$_2$ adrenoceptors [8]. Characterization of alpha-adrenoceptor subtypes has not yet been performed in brown fat, and species differences have to be considered.

In summary, the present results suggest the presence in brown adipocytes of alpha$_1$ adrenoceptors whose activation is responsible for the increase in the incorporation of [^{32}P]P$_i$ into phosphatidylinositol and phosphatidic acid. The alpha$_1$-adrenergic effect is probably related to the depolarization observed by Fink and Williams [6] in brown fat.

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* This work was supported by United States Public Health Service Research Grants AM 10149 and AM 21470 from the National Institutes of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health.

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Biochemical Pharmacology, Vol. 29, pp. 3333–3336.

0006-2952/80/1215-3333 $02.00/0

Sulphydryl dependence of the inhibition of mitogen-induced human lymphocyte proliferation by sodium aurothiomalate

(*Received* 6 *November* 1979; *accepted* 21 *August* 1980)

Gold mercaptides have been used effectively in the treatment of rheumatoid arthritis for several decades [1]. Although the nature of their anti-inflammatory activity *in vivo* is not known, gold compounds have been shown to inhibit lysosomal enzymes [2, 3], prostaglandin synthesis [4], γ-globulin aggregation [5], macrophage phagocytosis [6] and lymphocyte blastogenesis [7–9] among other biological processes *in vitro*. The activity of such compounds,