Table 2. Effect of metabolic inhibitors on uptake of [^{14}C]PCZ by L5178Y lymphoblasts *in vitro**

Metabolic inhibitor	Conc (M)	Per cent of control uptake (mean ± S. E.) 15 sec	10 min
IAA	1×10^{-4}	107.2 ± 1.8	106.4 ± 2.9
NEM	5×10^{-6}	98.8 ± 6.9	102.8 ± 6.6
POMB	5×10^{-5}	100.0 ± 3.6	87.8 ± 1.9
NaCN	1×10^{-4}	87.7 ± 3.6	90.5 ± 5.5
Oligomycin	5×10^{-6}	95.1 ± 1.0	116.4 ± 2.0
CCCP	1×10^{-5}	102.4 ± 4.3	93.4 ± 4.5
DNP	1×10^{-4}	104.4 ± 5.6	99.3 ± 5.1
	1×10^{-3}	102.6 ± 3.9	118.9 ± 3.1†

*Cells were incubated with metabolic inhibitors for 15 min before [^{14}C]PCZ was added and uptake of radioactivity at 15 sec and 10 min was compared in the presence and absence of inhibitors. Results are expressed as a percentage of control cell/medium radioactivity distribution ratio and were statistically evaluated by Student's two-tailed '*t*'-test. Each value represents the mean ±S.E. of four to eight determinations.

†$P < 0.01$;all other results were not statistically significant.

[^{14}C]PCZ by cells incubated in a balanced salt solution described by Martin[22] was compared with uptake in the same solution in which NaCl was replaced by an equivalent amount of either Tris, choline chloride or KCl. Uptake of 5×10^{-5} M [^{14}C]PCZ by L5178Y cells at 10 min in these solutions was essentially identical.

The findings that the rate of PCZ uptake was of the order of magnitude expected for a simple diffusion system and, further, not highly temperature dependent, that the cell/medium distribution ratio of free intact drug was less than unity, that uptake was non-saturable, was unaffected by several metabolic inhibitors, and was sodium-insensitive, all suggest that uptake of PCZ by L5178Y cells *in vitro* occurs by simple diffusion.

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Comparison of isoproterenol, salbutamol and talzolol as lipolytic agents with isolated rodent adipocytes

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Lands *et al.*[1] presented evidence that β-adrenergic receptors could be subdivided into β_1- and β_2-receptors according to their stimulatory activity on the heart and their bronchodilator action respectively. Using a series of agonists, they found a high correlation between stimulation of the heart and of lipolysis in the rat and concluded that the β-adrenergic receptor mediating lipolysis was of the β_1-type. Recent work[2, 3] has challenged this conclusion. Part of the evidence against a β_1-receptor in rat adipose tissue is that salbutamol is an

effective lipolytic agent[2, 4]. Salbutamol has been shown to be a selective β_2-receptor agonist[5]. Tazolol, on the other hand, has been shown to have preferential β_1-activity[6]. Tazolol has not been previously examined as a lipolytic agent in the rat, but plasma free fatty acid levels increased after tazolol administration to cats[7]. The present investigation compares the lipolytic efficacy of salbutamol and tazolol with that of isoproterenol in isolated epididymal adipocytes of the rat and the mouse.

Male rats (140–180 g) and mice (20–30 g) were obtained from ARS Sprague-Dawley, Madison, WI, and were held for at least 1 week, with *ad lib.* access to lab chow prior to use. Isoproterenol · HCl was obtained from Aldrich Chemical Co., Milwaukee, WI, salbutamol sulfate from Allen & Hanbury, Ltd., Ware, Herts, United Kingdom, and tazolol (1-isopropylamino-3-[2-thiazoloxy]-2-propanol) hydrochloride from Syntex Laboratory, Palo Alto, CA. All agonists were racemic mixtures. Crude collagenase, type CLS, was obtained from Worthington Biochemicals, Freehold, NJ. Bovine serum albumin Fraction V was obtained from Miles Research Laboratories, Elkhart, IN. All other enzymes and nucleotides were obtained from Sigma Co., St. Louis, MO. Both rats and mice were killed by cervical dislocation and the epididymal fat pads were removed. Isolated fat cells were prepared from pooled pads of three to six animals, as described by Lech and Calvert[8]. Incubations were performed at 37° in Krebs–Henseleit phosphate buffer, pH 7.4, with 4% bovine albumin under 95% O_2–5% CO_2. Test incubations were conducted for 45 min. Lipolysis was determined by measuring glycerol efflux enzymatically, as described by Wieland[9]. Results were expressed on the basis of adipocyte protein determined by the method of Ross and Schatz[10] on an aliquot of cell suspension.

Statistical significance was evaluated by analysis of variance and differences between treatment compared using Duncan's multiple range test[11].

The response of rat and mouse epididymal adipocytes to the three agonists evaluated in this study further supports the hypothesis that the β-adrenergic receptor mediating lipolysis in the rat is of the β_2-type. Furthermore, the results suggest that a similar situation occurs with mouse adipocytes. With rat adipocytes, it can be seen that isoproterenol was more potent than salbutamol but both possessed similar effects (Fig. 1). Tazolol, on the other hand, was not significantly active in concentrations as high as 10^{-3} M. Similar results were found with mouse adipocytes (Fig. 2). Again, the lipolytic response was similar to isoproterenol and salbutamol, although larger doses of the latter were required. In six experiments with mice (data not shown), no increase in basal lipolysis was found with concentrations of tazolol of 10^{-4} and 10^{-3} M. With adipocytes from mice and rats, salbutamol was maximally active in the range of 10^{-5} to 10^{-4} M. Higher concentrations than these produced no further increases in lipolysis (data not shown). Salbutamol has been shown previously to be lipolytically effective in rat adipose tissue by Grana *et al.*[2] and Fain *et al.*[4]. The present data confirmed those findings but also extended them to the mouse adipocytes. Harms *et al.*[3] have questioned the β_1-classification of the adrenergic lipolytic response in rats. They base their doubt on a comparison of pA_2 values in rat adipocytes obtained with a number of β-adrenergic blockers and those obtained with guinea pig atrial and tracheal preparations.

However, Frisk-Holmberg and Östman[12] have recently concluded that the adrenergic receptor mediating lipolysis in human adipose tissue is of the β_1-type, based on effects of the various receptor agonists and antagonists on glycerol release. In contrast, Kather and Simon[13] reported that cardioselective β-blocking agents of the β_1-type were less potent than the non-selective β-blockers in the isoproterenol activation of

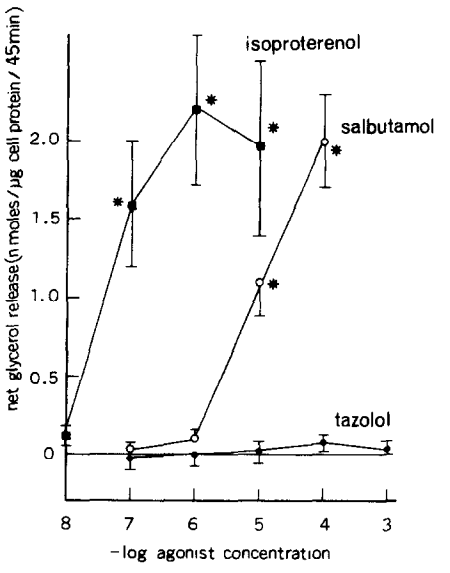

Fig. 1. Comparison of isoproterenol, salbutamol and tazolol on lipolysis in isolated rat adipocytes. Net stimulation of glycerol efflux by these agents is shown with the basal glycerol efflux (0.14 ± 0.05 nmole glycerol/μg of adipocyte protein/45 min) subtracted. Results are given as the mean ±S. E. of five to seven separate adipocyte preparations. Every agonist concentration, where tested, was incubated in duplicate with the respective adipocyte preparation, derived from epididymal fat pads pooled from three to six rats. The asterisk (*) signifies $P < 0.01$, compared to basal lipolysis by analysis of variance and Duncan's multiple range test.

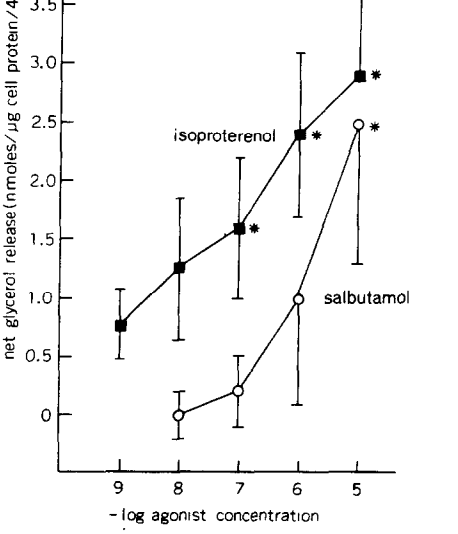

Fig. 2. Comparison of isoproterenol and salbutamol on lipolysis in isolated mouse adipocytes. Net stimulation of glycerol efflux by these agents is shown with the basal glycerol efflux (0.41 ± 0.30 nmole/μg of adipocyte/45 min) subtracted. Results are given as the mean ±S. E. of four to six separate adipocyte preparations. Every agonist concentration, where tested, was incubated in duplicate with the respective adipocyte preparation, derived from epididymal fat pads pooled from three to six mice. The asterisk (*) signifies $P < 0.01$, compared to basal lipolysis by analysis of variance and Duncan's multiple range test.

adenylate cyclase present in human fat cell ghosts. These workers concluded from these and previous findings that the β_1-type receptors of human adipose tissue differ from cardiac receptor sites. The complexity of generalizing the findings obtained under a particular set of conditions to a uniform β-receptor theory in adipose tissue is attributable to many variables including age, nutrition, and the site of obtaining the adipose tissue. Kather *et al.*[14] recently reported that adenylate cyclase in membrane preparations from abdominal adipose tissue in humans was more sensitive to stimulation by adrenaline than was adenylate cyclase in adipocyte ghosts from the gluteal region of the same person.

Tazolol has been shown to produce an increase in plasma free fatty acids in the cat[7]. More recently, Clark and Poyser[15] have concluded that the selectivity of tazolol is of the β_1-type, based on data obtained with isolated tissue preparations of both the guinea pig and rat. However, it is clear from the data presented herein that tazolol is without lipolytic activity in adipocytes from epididymal fat pads of rats and mice. These data and those recently presented by Kather and Simon[13] further emphasize the difficulties in classifying the lipolytic response of adipose tissue to β-agonists as originally discussed by Himms-Hagen[16].

In summary, three adrenergic agonists (isoproterenol, salbutamol and tazolol) of differing receptor specificity have been examined as lypolytic agents with isolated rat and mouse adipocytes. With both species, salbutamol, a β_2-selective agonist, showed lipolytic activity similar to that of isoproterenol although the latter was considerably more potent. Tazolol, a specific β_1-agonist was without lipolytic activity. These results would suggest that the β-receptor involved in lipolysis is of the β_2-type in the rat and mouse.

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