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LETTERS TO THE EDITOR

The effect of butoxamine, *N*-isopropylmethoxamine and salbutamol (AH-3365) on melanophore β-adrenergic receptors

Recently, Goldman & Hadley (1969) demonstrated that the melanophores of the lizard, *Anolis carolinensis*, possess β-adrenergic receptors which mediate melanosome (melanin granule) dispersion within melanophores in response to adrenergic stimulation. Melanosome dispersion within melanophores is responsible for the normal darkening of skin in *Anolis*. Salbutamol (AH-3365) has been reported to be a new selective β-adrenergic receptor stimulant (Brittain, Farmer & others, 1968; Cullum, Farmer & others, 1969) which has a "considerably greater action on bronchial smooth muscle than other smooth muscles affected by β-stimulants" (Brittain & others, 1968). Similarly, the β-adrenergic blocking agents, *N*-isopropylmethoxamine and butoxamine, are selective in that they block "some but not all of the β-adrenergic responses to catecholamines" (Wilkenfeld & Levy, 1968). We have, therefore, tested these agents to determine whether they have any activity on the β-receptors of *Anolis* melanophores.

Skins of *Anolis carolinensis* were removed and prepared (Goldman & Hadley, 1969). Responses to pharmacological stimulation were monitored as changes in light reflectance from the epidermal surface of skins as originally described for the frog skin bioassay for melanophore stimulating hormone, MSH (Shizume, Lerner & Fitzpatrick, 1954). An increase in reflectance indicated skin lightening resulting from melanosome aggregation whereas a decrease in reflectance indicated skin darkening caused by melanosome dispersion within melanophores. The preparation and method of adding pharmacological agents has been described (Goldman & Hadley, 1969).

Table 1. *Effect of salbutamol on melanophore β receptors*

Exp.	No. of animals	Treatment M	% Decrease in reflectance*
A	8	Isoprenaline 1×10^{-6}	16 ± 2·18
	8	Salbutamol 1×10^{-5}	5 ± 2·56
B	6	Isoprenaline 1×10^{-6}	25 ± 1·71
	7	Salbutamol 1×10^{-4}	10 ± 2·08

* Values are means ±s.e. Decrease in reflectance represents skin darkening. The results are the greatest response within 60 min after addition of salbutamol and isoprenaline. In both experiments the difference between the groups is significant ($P < 0.01$ in A; $P < 0.001$ in B) according to the student's *t*-test.

Salbutamol darkens the skins of *Anolis* but this response is less than the isoprenaline-induced darkening (Table 1). Even at a concentration of 10^{-4}M salbutamol darkened the skins much less than isoprenaline which was at a concentration of 10^{-6}M. These observations agree with the findings of Brittain & others (1968) and Cullum & others (1969) who found salbutamol (AH-3365) to be a selective stimulant of β-adrenergic receptors that was less potent *in vitro* than isoprenaline.

Using the β-adrenergic blocking agents, we found (Fig. 1) that even at concentrations of 10^{-4}M neither *N*-isopropylmethoxamine nor butoxamine inhibited darkening by isoprenaline. In fact, if anything, these agents seemed to slightly enhance the

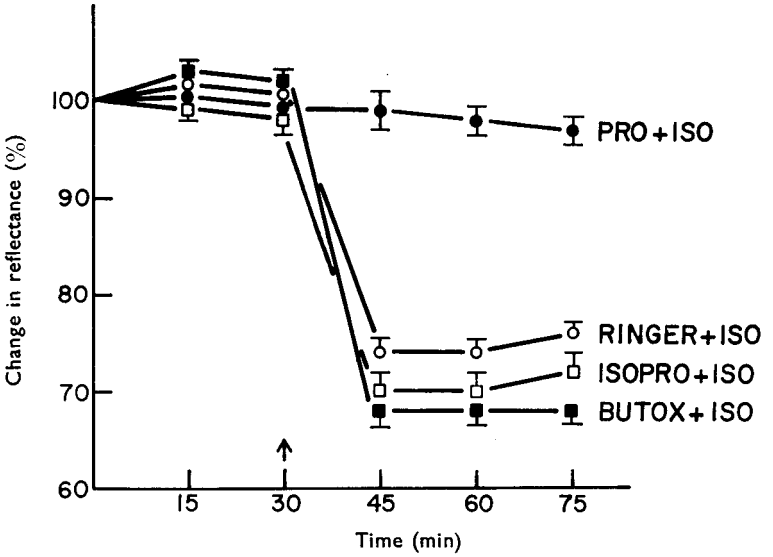

FIG. 1. Inhibition of isoprenaline-induced darkening by β-adrenergic blocking agents. Three groups of eight skins each were incubated in β-blocking agents ●, propranolol (PRO) 1×10^{-4}M; □, *N*-isopropylmethoxamine (ISOPRO) 1×10^{-4}M; ■, butoxamine (BUTOX) 1×10^{-4}M); while one group of eight skins remained in Ringer solution (○). After 30 min (arrow) isoprenaline (ISO, 1×10^{-6}M) was added to all of the groups. Each point on the graph represents the mean reflectance measurement from the eight skins per group. Vertical lines indicate the standard error of the mean.

darkening. Propranolol, however, completely blocked isoprenaline-induced darkening. Again, our experiments agree with the findings by Levy (1964, 1966) and Wilkenfeld & Levy (1968) that *N*-isopropylmethoxamine and butoxamine inhibit only some β-adrenergic receptor responses and are different, therefore, from other β-adrenergic blocking agents such as propranolol which inhibit all β-adrenergic receptor stimulation.

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