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The effect of prenylamine on adrenaline-induced hypercholesterolemia in mice

The administration of adrenaline causes a rise in serum cholesterol level in many animal species. The hypercholesterolemia is preceded by an elevation of free fatty acid levels in rats (Shafrir, Sussman & Steinberg, 1960; Shafrir & Steinberg, 1960), dogs (Kaplan, Stafford & Gant, 1957; Shafrir, Sussman & Steinberg, 1959), and rabbits (Dury, 1957), and it seems that the rise in serum cholesterol is secondary to the rise in blood free fatty acids (Gidez, Roheim & Eder, 1962; Nestel & Steinberg, 1963; Steinberg, 1963). We now report the effect of adrenaline on cholesterol serum levels in mice, and the modulation of this effect by prenylamine and other adrenergic blocking drugs known to inhibit the free fatty acid response to adrenaline.

Swiss-Webster (ICR) albino male mice (Harlan Industries, Cumberland, Indiana) 25–28 g were housed in groups of five at 25 $\pm$ 3° with freely available food and water. All injections were subcutaneous at varying sites in the dorsal region. The drugs were dissolved or suspended in sesame oil in a volume of 10 ml/kg weight. The doses as base of drug were: (−)-adrenaline, 1 mg/kg; prenylamine, 5, 10, 25 and 50 mg/kg; propranolol, 10 mg/kg; phenoxybenzamine hydrochloride, 1 mg/kg; and phentolamine hydrochloride, 10 mg/kg. The schedule for drug administration was: blocking drug at 5 30 am on days 1, 2 and 3 and again 12 h later on days 1 and 2. Adrenaline was given at 6 am on days 2 and 3 and 12 h later on day 2. Analysis was at 6 pm on day 3. In experiments where either adrenaline or prenylamine (25 or 50 mg/kg) was given alone, in injection of 10 ml/kg of sesame oil replaced the blocking drug; thus eight injections were always made in the three-day period. The controls similarly had only sesame oil. A group of older male mice, 32–36 g was also included.

Blood was obtained from the mice by decapitation, and serum cholesterol measured colorimetrically (Watson, 1960).

No difference was found between control serum levels of cholesterol of either weight groups of mice (Table 1). Whereas adrenaline administration caused no significant change in serum cholesterol level in the older mice (32–36 g), there was a statistically significant ($P < 0{\cdot}001$) elevation in mean cholesterol value above control

Table 1. *Serum cholesterol levels in male mice after various sympathomimetic and sympatholytic drugs*

No. of mice per group	Mice weight (g)	Treatment and dosage	Cholesterol serum levels (mg %) Mean ± s.e.
30	25–28	Sesame oil controls (10 ml/kg)	118 ± 2·7
10	32–36	Sesame oil controls (10 ml/kg)	115 ± 3·0
19	25–28	Adrenaline (1 mg/kg)	150 ± 4·0
20	32–36	Adrenaline (1 mg/kg)	126 ± 7·0
20	25–28	Prenylamine (50 mg/kg)	128 ± 5·4
20	25–28	Prenylamine (25 mg/kg)	121 ± 4·3
20	25–28	Prenylamine (25 mg/kg) + adrenaline (1 mg/kg)	140 ± 4·7
20	25–28	Prenylamine (10 mg/kg) + adrenaline (1 mg/kg)	125 ± 4·0
20	25–28	Prenylamine (5 mg/kg) + adrenaline (1 mg/kg)	140 ± 5·7
20	25–28	Propranolol (10 mg/kg) + adrenaline (1 mg/kg)	125 ± 4·5
18	25–28	Phenoxybenzamine (1 mg/kg) + adrenaline (1 mg/kg)	116 ± 3·8
20	25–28	Phentolamine (10 mg/kg) + adrenaline (1 mg/kg)	110 ± 1·9

level in the younger mice (25–28 g). This response was inhibited by pretreatment with phenoxybenzamine (1 mg/kg) and phentolamine (10 mg/kg), by propranolol (10 mg/kg), and by prenylamine (10 mg/kg). Pretreatment both with higher (25 mg/kg) and lower (5 mg/kg) doses of prenylamine failed to block adrenaline-induced hypercholesterolemia but did reduce the response slightly. In 10 mice pretreated with prenylamine, 50 mg/kg, four deaths occurred shortly after injection of adrenaline. Given alone, prenylamine, 25 and 50 mg/kg, did not raise serum cholesterol levels significantly above the control value ($P < 0{\cdot}1$). The mean weights of drug-treated animals did not differ from that of controls throughout.

Prenylamine differs in its effects from either of the two classes of blocking agents used in that it is reported to produce a reserpine-like depletion of catecholamine storage granules (Schöne & Lindner, 1960; Carlsson, Hillarp & Waldeck, 1963), and was shown to exhibit both α-adrenolytic (Kochsiek, Scheler & Bretschneider, 1960; Lindner, 1963; Kuschke, Eckmann & others, 1964; Obianwu, 1967) and β-adrenolytic (Lindner, 1960, 1964; Haas, 1964; Fleckenstein, Döring & others, 1968) properties. Lindner (1964) and Braunsteiner, Sailer & Sandhofer (1965) showed prenylamine to block the noradrenaline-induced mobilization of free fatty acids in dogs, and to lower free fatty acid blood levels in man. The slight elevation of cholesterol seen in our experiments after prenylamine, 25 and 50 mg/kg, as well as the failure of 25 mg/kg to block the hypercholesterolemic effect of adrenaline may reflect the sympathomimetic properties of prenylamine in discharging endogenous catecholamines. Moreover, the deaths occurring upon administration of adrenaline to the mice pretreated with prenylamine, 50 mg/kg, may arise from a failure of uptake into stores (Carlsson, Hillarp & Waldeck, 1963). Whereas a dose of 5 mg/kg was too low to effect adrenergic blockade, a dose of 10 mg/kg blocked the adrenaline-induced rise of serum cholesterol. It is therefore apparent that at lower doses of prenylamine the sympathetic blocking properties of this drug supervene, whereas at higher doses the sympathomimetic properties become more prominent and overcome the intrinsic blocking effect.

The failure to elicit a rise in serum cholesterol after adrenaline administration to older mice remains a matter of observation only. A similar age dependent effect

of catecholamines on free fatty acid mobilization was observed in the rat by Jelinkova & Hruza (1963, 1964).

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Estimation of drug metabolite elimination kinetics in man by the synthesis-blocking method

It is often necessary for the complete characterization of the pharmacokinetics of a drug (Levy, Amsel & Elliot, 1969) and for drug biotransformation interactions (Amsel & Levy, 1969; Amsel & Levy, 1970) to measure the elimination (or excretion) rate constants of drug metabolites. Many drug metabolites are not readily synthesized or available commercially; they may be unstable in or not well absorbed from the gastrointestinal tract (Levy, Amsel & Elliott, 1969; Levy, Weintraub & others, 1966) and unsuitable for parenteral administration. The elimination rate constants of such metabolites, which include most glucuronides and sulphates, cannot be determined by administering the metabolite as such. Some mathematical techniques have been developed to estimate the rate constants indirectly from the urinary excretion rates of free drug and metabolites (Cummings, Martin & Park, 1967; Martin, 1967) but these estimations are difficult or impossible if little or no drug is excreted in non-metabolized form (Cummings, King & Martin, 1967).