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SYMPATHOLYTIC DRUGS AND HYPERLIPAEMIA INDUCED IN RATS · BY INTRAPERITONEAL INJECTIONS OF SURFACE-ACTIVE AGENT

BY

R. BRUS, M. CHRUŚCIEL, T. L. CHRUŚCIEL AND A. PLECH

From the Silesian Academy of Medicine, Department of Pharmacology, Zabrze 8, Poland

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Hyperlipaemia and hypercholesterolaemia were induced in white rats by intraperitoneal injections of tyloxapol. Various sympatholytics and adrenolytics, including blocking agents of β-receptors, were given simultaneously with tyloxapol. Bretylium tosylate prevented the increase in the serum levels of esterified fatty acids and cholesterol caused by tyloxapol. Phentolamine decreased the enhancement by tyloxapol of cholesterol and total lipid concentrations in the serum. Guanethidine and phenoxybenzamine reduced the increase in the concentration of esterified fatty acids, whereas dichloroisoprenaline insignificantly increased the tyloxapol-induced hyperlipaemia. A small dose of pronethalol slightly increased the esterified fatty acid level in tyloxapol-treated animals ; a large dose significantly decreased the serum cholesterol concentration.

Kellner, Correll & Ladd (1951) observed hypercholesterolaemia, hyperlipidaemia, and hyperphosphatidaemia in rabbits caused by a single intraperitoneal injection of tyloxapol (Superinone, Triton WR-1339, a polymer of p-*iso*-octylpolyoxyethylenephenol and formaldehyde). This effect upon serum lipids has been confirmed in mice by Cornforth, D'Arcy Hart, Rees & Stock (1951), and in rats by Friedman & Byers (1953) and others (Schön & Berg, 1957 ; Rosenman, Breall, Byers & Rabin, 1959), but the mechanism of action is not yet completely elucidated. It is known that tyloxapol interferes with lipoprotein lipase (Schotz, Scanu & Page, 1957) and increases cholesterol biosynthesis *in vitro* (Frantz & Hinkelman, 1955 ; Ballardi, Paoletti & Paoletti, 1958 ; Bucher, McGarrahan, Gould & Loud, 1959 ; Scarselli & Fossati, 1961). Garattini, Morpurgo, Paoletti & Paoletti (1959) have proposed that tyloxapol hypercholesterolaemia might be used as a test for the evaluation of the hypocholesterolaemic properties of various drugs.

Many adrenotropic agents increase serum levels of cholesterol and free fatty acids (Kaplan & Gant, 1955 ; Engelberg, 1959). Schotz & Page (1960), Pilkington, Love, Robinson & Titterington (1962) and Wenke, Mühlbachová & Hynie (1962) have shown that various adrenolytic drugs prevent this action of catechol amines upon lipid metabolism. We were interested in assaying the influence of various sympatholytic and adrenolytic drugs upon tyloxapol-induced hyperlipaemia.

METHODS

We have followed the procedure of Garattini *et al.* (1959). Adult male white rats (mean weight 150 g) were starved for approximately 18 hr and then injected intraperitoneally with an aqueous solution of tyloxapol in a dose of 200 mg/kg. The drugs investigated were injected together with tyloxapol in groups of at least five rats. In each experiment appropriate control groups were run. After 18 hr samples of blood were taken by heart puncture during light narcosis with ether, and the following biochemical analyses of the serum were performed: cholesterol (Turner & Eales, 1957; Henly, 1957), total lipids (Swahn, 1953) and esterified fatty acids (Stern & Shapiro, 1953). The results were submitted to statistical analysis using Student's *t*-test. Values of *P* below 0.02 were regarded as significant.

RESULTS

Tyloxapol caused a remarkable increase of the serum levels of cholesterol, total lipids and esterified fatty acids. It should be pointed out that although in all rats the responses to tyloxapol were in the same direction, the individual sensitivities of the animals were different, causing large variations in the degree of response. Various sympatholytic and adrenolytic drugs influenced the effect of tyloxapol upon serum lipids. Detailed results of one of the experiments are shown in Fig. 1, and a summary of all the results is presented in Table 1.

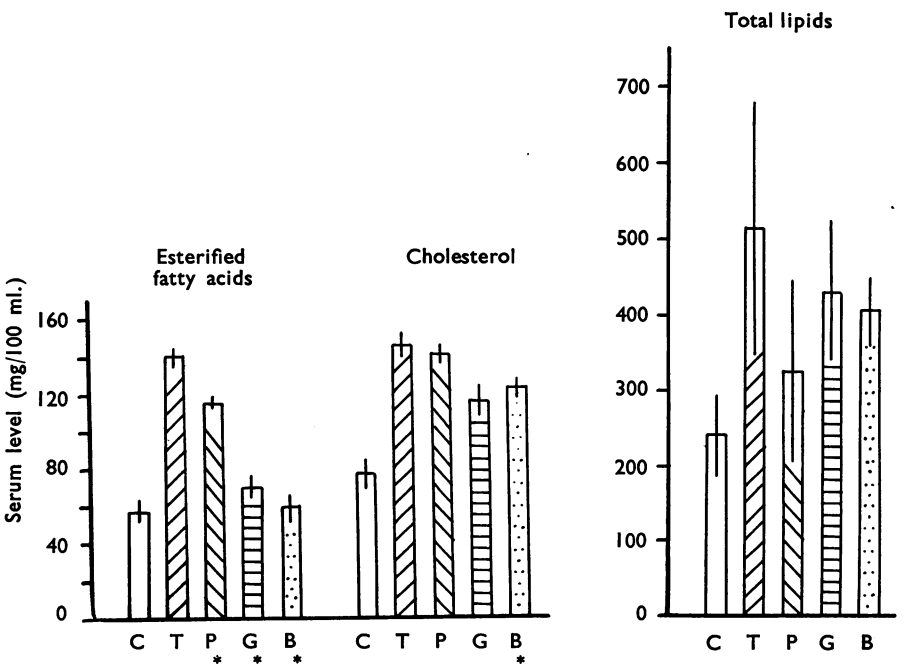

Fig. 1. Influence of phenoxybenzamine, guanethidine and bretylium on tyloxapol-induced changes in serum lipids. Expt. no. 1. Ordinates: serum levels of esterified fatty acids, cholesterol and total lipids. C=control, no treatment; T=tyloxapol, 200 mg/kg; P=tyloxapol+phenoxybenzamine, 40 mg/kg; G=tyloxapol+guanethidine, 40 mg/kg; B=tyloxapol+bretylium tosylate, 40 mg/kg. Vertical lines represent standard errors of means. Asterisks indicate statistical significance ($P>0.02$) of the difference from the tyloxapol only values (T).

TABLE 1

THE INFLUENCE OF SYMPATHOLYTIC AND ADRENOLYTIC DRUGS UPON TYLOXAPOL-INDUCED INCREASE IN THE SERUM LEVEL OF ESTERIFIED FATTY ACIDS, CHOLESTEROL AND TOTAL LIPIDS IN WHITE RATS

Tyloxapol was injected intraperitoneally in a dose of 200 mg/kg. E.F.A.=esterified fatty acids. The methanesulphonate of dihydroergotamine was used. 0=No change, or change less than 10%; +, −=change greater than 10%; ++, −−=change greater than 20%; and +++, −−−= statistically significant change

Expt. no.	No. of rats	Treatment	Dose (mg/kg)	Change of tyloxapol-induced increase in serum concentration of: E.F.A.	Cholesterol	Total lipids
Sympatholytic drugs						
I	5	Guanethidine	40	−−−	−	−
II	6	Bretylium tosylate	10	0	−	−−
I	5	Bretylium tosylate	40	−−−	−−−	−
V	8	Bretylium tosylate	40	−−−	−	0
Blocking drugs for α-receptors						
VI	10	Phentolamine	4		−	
II	5	Phentolamine	40	−	−−	−−
IV	5	Phentolamine	40	−−	−−−	−−−
II	6	Dihydroergotamine	4	−−	0	0
V	8	Dihydroergotamine	4	−	−−	−
I	5	Phenoxybenzamine	40	−−−	0	−−
Blocking drugs for β-receptors						
II	6	Dichloroisoprenaline	4	+	++	0
V	9	Dichloroisoprenaline	40	0	++	++
IV	6	Pronethalol	4	+	0	0
III	6	Pronethalol	40	0	−−−	−−
V	9	Pronethalol	40	−	0	0
VI	10	Pronethalol	40		−−−	
VII	10	Pronethalol	40	0		+

Bretylium tosylate (Darenthin), in a dose of 40 mg/kg, decreased the level of cholesterol, completely prevented any rise in the esterified fatty acids and decreased the tyloxapol hyperlipaemia in the serum. The decrease of total lipid concentration was not statistically significant because of the large variations in individual results. A lower dose of bretylium (4 mg/kg) caused similar although insignificant effects.

Guanethidine (Ismelin), in a dose of 40 mg/kg, also decreased the effect of tyloxapol upon the serum level of esterified fatty acids and insignificantly diminished the concentration of cholesterol and total lipids.

Phentolamine (Regitin), in a dose of 40 mg/kg, and phenoxybenzamine (Dibenzyline), in the same dose, greatly reduced the effects of tyloxapol upon the serum lipid fractions investigated. The effect of phentolamine upon cholesterol and total lipid levels and the effect of phenoxybenzamine upon esterified fatty acids were statistically significant. It should be noted that, even when insignificant, depressions were present in most experiments. Dihydroergotamine methylsulphonate, in a dose of 4 mg/kg, insignificantly reduced the effects of tyloxapol.

A standard β-receptor blocking drug, dichloroisoprenaline, given in doses of 4 and 40 mg/kg, did not decrease the influence of tyloxapol. On the contrary, the increase of esterified fatty acid, total lipid and especially cholesterol serum concentrations was greater (although insignificantly) when dichloroisoprenaline was injected

with tyloxapol. A small dose of pronethalol (Nethalide, 4 mg/kg) also slightly increased the tyloxapol-induced esterified fatty acid level and did not decrease the influence of tyloxapol upon concentrations of cholesterol and total lipids. But a large dose (40 mg/kg) of pronethalol in most experiments caused a statistically significant decrease of the serum concentration of cholesterol which had previously been increased by tyloxapol. The effect upon the level of esterified fatty acids was much less, even insignificant, and the level of the total lipids was not significantly modified by pronethalol. In one experiment the level of the free fatty acids in the serum was analysed by the method of Grossman, Stadler, Cushing & Palm (1955): tyloxapol increased it considerably ($P<0.05$), but pronethalol (40 mg/kg) did not influence this increase.

DISCUSSION

The inhibition of some effects of tyloxapol *in vivo* by the sympatholytic drugs bretylium and guanethidine, by phenoxybenzamine and phentolamine, blocking substances for α-receptors, and by large doses of the β-blocking drug, pronethalol, may be compared with some recent observations of Wenke *et al.* (1962). They have reported that the effects of the α-sympathomimetic noradrenaline, the β-sympathomimetic isoprenaline, and the α- and β-sympathomimetic drug adrenaline upon lipid release from adipose tissue are blocked *in vitro* by the α-sympathetic depressant phentolamine and the β-sympathetic depressant drug dichloroisoprenaline. In our experiments phentolamine, bretylium and pronethalol in sufficiently large doses decreased the effect of tyloxapol upon serum cholesterol *in vivo* in white rats, while guanethidine and smaller doses of these drugs showed less effect. Dichloroisoprenaline injected with tyloxapol increased its effect upon serum cholesterol concentration by over 20%. This effect of dichloroisoprenaline is most probably due to its known adrenomimetic properties. Love, Carr & Ashmore (1963) have reported recently that dichloroisoprenaline *in vivo* enhances the net production of free fatty acids in the rat. Dichloroisoprenaline in the doses applied apparently behaves similarly to adrenaline. The increase in plasma free fatty acids caused by dichloroisoprenaline is due to an accelerated release from adipose tissue. It is tempting to assume that in our experiments the increase of cholesterol, esterified fatty acids and total lipids was greater in experiments with dichloroisoprenaline because this drug released not only free fatty acids but also other lipids from adipose tissue.

The influence of the sympatholytic and adrenolytic drugs upon the serum levels of esterified fatty acids and total lipids in tyloxapol-treated rats brought to light further differences between the β-sympathetic depressants dichloroisoprenaline and pronethalol and the other drugs tested.

The sympatholytics and α-sympathetic depressants used in our experiments had a marked influence upon the effects of tyloxapol. Both the sympatholytics, guanethidine and bretylium, significantly decreased the level of esterified fatty acids. Phenoxybenzamine also decreased this level significantly, and the other α-sympathetic depressants phentolamine and dihydroergotamine acted similarly but in an insignificant manner. The effect of both β-sympathetic depressants upon the

esterified fatty acids was parallel with their effect upon the cholesterol level but much less conspicuous and statistically insignificant.

The effect of sympatholytics and adrenolytics upon the tyloxapol-induced increase of total lipid concentration in the serum was less prominent. Only phentolamine significantly decreased the level of total lipids but the changes caused by other drugs were similar to those in the cholesterol level, although smaller. Dichloroisoprenaline in a large dose increased the effect of tyloxapol upon the total lipids, whereas the addition of pronethalol to tyloxapol gave some controversial results.

The mechanism of the tyloxapol-induced hyperlipaemia needs further clarification. The observed effects of various drugs suppressing the activity of the sympathetic nerve endings upon fat metabolism at the cellular level seem to indicate that the mechanism of the tyloxapol-induced hyperlipaemia is in some way, most probably indirect, connected with adrenomimetic adrenergic mechanisms involved in fat metabolism. However, we cannot exclude the possibility that the effect of tyloxapol is connected only with a back reaction on fatty acid mobilization.

The possibility of the existence of a special catechol amine receptor triggering or at least modifying the fat metabolism at the cellular level, as postulated by Wenke *et al.* (1962), is supported by our results. Some β-adrenergic blocking drugs devoid of adrenomimetic properties, but more potent than those at present available, would be useful to define the character of this receptor.

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