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The inhibitory effect of sulphonylurea derivatives on liver glycogenolysis increased by catecholamines

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Hypoglycaemic sulphonylureas inhibit the increased glycogenolysis and glucose release of the rat perfused liver produced by β-adrenergic agonists. The β-receptor antagonist propranolol exerted a similar effect. Dichloroisoprenaline increases the hypoglycaemic effect of chlorpropamide given in doses ineffective in the intact rat. Hypoglycaemic sulphonylureas have no effect on the catecholamine sensitivity of the nictitating membrane and heart auricle of the cat, indicating that their ability to inhibit glycogenolysis induced by β-receptor agonists is not due to a β-receptor antagonist activity as is the action of propranolol.

Previously reported experiments from our laboratory have demonstrated that the rate of glucose release from the isolated perfused liver of rats is stimulated by adrenergic β-receptor agonists and that this effect is abolished by specific β-receptor antagonists (Vizi & Pogátsa, 1965; Vizi, Pogátsa & Káldor, 1965). Similarly perfusion of the liver with a glucose-free Tyrode solution augmented both glucose release and glycogenolysis and these actions were also inhibited by β-receptor blocking drugs. It is well known that one of the principal characteristics of the hypoglycaemic sulphonylureas is to decrease hepatic glucose release and glycogenolysis (Purnell, Arai & others, 1956; Ashmore, Cahill & others, 1958; Pogátsa & Káldor, 1965). Chrusciel, Janiec & Brus (1964) found the sulphonylurea derivative, chlorpropamide, to antagonize isoprenaline's action on the blood pressure of cat and in relaxing the cat uterus. On the other hand, according to de Divitiis, Giordano & others (1968) propranolol decreases the hypoglycaemic effect of tolbutamide in healthy subjects. Propranolol also increases the toxicity of insulin in the rat (Byers & Friedman, 1966) and delays the compensatory response to hypoglycaemia in man (Abramson, Arky & Woeber,1966) presumably due to antagonism of increased amounts of circulating adrenaline (Goldfien, Zileli & others, 1958). However, Calvey & Summerill (1968) observed in rabbits, that propranol decreased resting plasma glucose, augmented the effects of insulin, and delayed the compensatory response to hypoglycaemia. It seemed worthwhile to examine whether the sulphonylureas have any influence on the effect of isoprenaline or adrenaline on the carbohydrate metabolism of the liver, and whether the β-adrenergic blocking effect of the sulphonylureas can be unequivocally established or not.

EXPERIMENTAL

Methods

Isolated liver perfusion was by a modification (Vizi & others, 1965) of the method of Issekutz (1924). To examine the possible effect of β-adrenergic blockade on the

hypoglycaemic effect of sulphonylurea compounds, albino rats of either sex, 150–200 g, fasting for 8 h were given dichloroisoprenaline (7 mg/kg) and chlorpropamide (20 mg/kg) intraperitoneally. Blood samples for glucose determination (Hagedorn & Jensen, 1923) were taken from the tail vein of the animals before the administration of the compounds and after 1, 2 and 3 h.

The possible β-adrenergic blocking effect of the sulphonylureas was determined by the method of Smith (1963) and György, Molnár & Dóda (1965) on the nictitating membrane of the cat and on cat auricle suspended in Tyrode solution. The auricles were placed in 10 ml of Tyrode solution at 31°. The left auricle preparations were stimulated with a frequency of 120/min using square waves at twice the threshold voltage. For right auricle preparations the increases in heart rate caused by isoprenaline were recorded as well as the isotonic myocardium contractions. We wanted to know whether or not the sulphonylureas had any influence on the positive inotropic and chronotropic effect of $2{\cdot}85 \times 10^{-8}$ M isoprenaline. The compounds in 0·2 ml volume were placed in the organ bath to give a bath concentration of $3{\cdot}69 \times 10^{-3}$ M 3–10 min before the administration of isoprenaline which was administered at 20–25 min intervals. In these experiments cats of either sex, 2·5–3 kg were used.

Statistical significance was analysed by Student's *t*-test.

Drugs. Adrenaline tartrate (Burroughs Wellcome). (—)-Isoprenaline bitartrate (Isolevin, Cilag-Chemie). Propranolol hydrochloride (Inderal, ICI). Dichloroisoprenaline hydrochloride (DCI, Boehringer). Chlorpropamide (Diabinese, Pfizer). Carbutamide (Nadisan, Boehringer). N_1-Sulphanilyl-N_2-methoxypropyl-carbamide (AH 6848, Boehringer). Insulin (Gedeon Richter). The concentrations are expressed in molar terms.

RESULTS

The rate of glucose release from perfused rat liver was significantly potentiated by removing glucose from the perfusing Tyrode solution. Similarly, there was a greater fall in hepatic glycogen content. When carbutamide or chlorpropamide, $1{\cdot}85 \times$

Table 1. *Perfusion of the isolated liver of the rat. Alterations of glucose release, glycogen concentration into normal Tyrode solution or into glucose-free Tyrode solution, after the administration of various concentrations of carbutamide, chlorpropamide, AH 6848 compound or insulin in glucose-free solution*

		mg glucose/g wet liver h^{-1} (mean ± s.e.) at times (min)		Change of glycogen content
		30	120	%
Tyrode solution	(10)	10·2 ± 1·5	4·2 ± 0·9	—49 ± 9
Glucose-free Tyrode solution	(10)	18·4 ± 2·3[1]	6·7 ± 1·4	—76 ± 4[1]
Carbutamide, $1{\cdot}86 \times 10^{-3}$ M	(10)	16·1 ± 2·2	7·6 ± 0·9	—66 ± 5
Carbutamide, $3{\cdot}72 \times 10^{-3}$ M	(10)	11·1 ± 1·1[3]	3·1 ± 0·9[2]	—58 ±4[3]
Chlorpropamide, $1{\cdot}86 \times 10^{-3}$ M	(10)	14·8 ± 1·9	6·9 ± 1·0	—64 ± 6
Chlorpropamide, $3{\cdot}72 \times 10^{-3}$ M	(10)	9·3 ± 1·6[3]	4·7 ± 0·9[2]	—61 ± 6[2]
AH 6848 comp., $3{\cdot}48 \times 10^{-3}$ M	(6)	17·3 ± 1·5	8·5 ± 1·3	—79 ± 5
Insulin 0·02 U/100 ml	(10)	16·1 ± 3·0	7·8 ± 1·8	
Insulin 0·4 U/100 ml	(7)	6·2 ± 2·8[3]	1·4 ± 1·6[2]	

Carbutamide, chlorpropamide, AH 6848 compound and insulin were each given at the beginning of perfusion. Number in brackets indicate the number of perfusions. The significance of the corresponding solutions with respect to the normal Tyrode [1]$P < 0{\cdot}02$. The significance of the corresponding solutions with respect to the glucose-free Tyrode [2]$P < 0{\cdot}05$; [3]$P < 0{\cdot}01$.

10^{-3} M, were added to glucose-free Tyrode there was no significant change in glucose loss although at twice this concentration both measurements were significantly reduced (Table 1). The chemically related AH 6848 which is devoid of hypoglycaemic activity, was ineffective. Insulin markedly inhibited the loss of glucose.

In the presence of glucose (in Tyrode solution), both carbutamide and chlorpropamide significantly antagonized the glucose release caused by isoprenaline. Propranolol and insulin were also effective in preventing glucose release, so much so that there appeared to be a net uptake of glucose from the perfusate into the liver. AH 6848 was again inactive. Qualitatively similar results were obtained when adrenaline was substituted for isoprenaline (Table 2).

Table 2. *Perfusion of the isolated liver of the rat. Alterations of glucose release and glycogen concentration after the administration of isoprenaline, adrenaline and various concentrations of carbutamide, chlorpropamide, AH 6848 compound, insulin and propranolol in normal Tyrode solution*

		mg glucose/g wet liver h^{-1} (mean ± s.e.) at times (min)		Change of glycogen content
		30	120	%
Tyrode solution	(10)	10·2 ± 1·5	4·2 ± 0·9	—49 ± 9
(—)-Isoprenaline, $5{\cdot}7 \times 10^{-8}$ M	(10)	11·9 ± 1·8	10·9 ± 2·3[1]	—89 ± 3[3]
Carbutamide, $0{\cdot}37 \times 10^{-3}$ M and (—)-isoprenaline	(10)	10·2 ± 0·9	4·2 ± 0·5[5]	—62 ± 11[4]
Carbutamide, $3{\cdot}72 \times 10^{-3}$ M and (—)-isoprenaline	(10)	3·6 ± 4·4[4]	1·1 ± 1·7[6]	—48 ± 7[7]
Chlorpropamide, $0{\cdot}37 \times 10^{-3}$ M and (—)-isoprenaline	(10)	9·5 ± 1·3	5·0 ± 0·8[4]	—66 ± 6[6]
Chlorpropamide, $3{\cdot}72 \times 10^{-3}$ M and (—)-isoprenaline	(10)	8·6 ± 1·4	2·8 ± 1·2[5]	—60 ± 9[6]
AH 6848 comp., $3{\cdot}48 \times 10^{-3}$ M and (—)-isoprenaline	(10)	11·5 ± 1·5	11·1 ± 1·5	—80 ± 3
Insulin, 0·02 U/100 ml and (—)-isoprenaline	(10)	13·2 ± 2·9	15·6 ± 2·3	—59 ± 5[6]
Insulin, 0·4 U/100 ml and (—)-isoprenaline	(11)	9·2 ± 2·6	—2·7 ± 1·7[7]	—10 ± 24[6]
Propranolol, $2{\cdot}38 \times 10^{-5}$ M and (—)-isoprenaline	(10)	8·0 ± 3·4	—1·9 ± 1·2[7]	—54 ± 35[6]
Adrenaline, $6{\cdot}6 \times 10^{-7}$ M	(10)	11·1 ± 2·5	9·9 ± 1·4[1]	—84 ± 5[2]
Carbutamide, $0{\cdot}37 \times 10^{-3}$ M and adrenaline	(10)	13·3 ± 0·9	0·9 ± 0·7[9]	—37 ± 4[9]
Carbutamide, $3{\cdot}72 \times 10^{-3}$ M and adrenaline	(10)	3·7 ± 0·7[8]	1·3 ± 0·3[9]	—49 ± 6[9]
AH 6848, $3{\cdot}48 \times 10^{-3}$ M and adrenaline	(6)	10·9 ± 0·7	10·0 ± 0·7	—82 ± 5
Insulin, 0·02 U/100 ml and adrenaline	(6)	16·3 ± 2·3	14·3 ± 2·9	—57 ± 9
Insulin, 0·4 U/100 ml and adrenaline	(6)	9·6 ± 5·2	0·5 ± 0·6[9]	—23 ± 18[8]

(—)-Isoprenaline, $5{\cdot}7 \times 10^{-8}$ M and adrenaline, $6{\cdot}6 \times 10^{-7}$ M were each given 30 min after the beginning of the perfusion and carbutamide, chlorpropamide AH 6848, insulin and propranolol were each given at the beginning of the perfusion. Numbers in brackets indicate the number of perfusions. The significance of the corresponding solutions with respect to normal Tyrode $^{1}P < 0{\cdot}02$; $^{2}P < 0{\cdot}01$; $^{3}P < 0{\cdot}001$. Significance of the corresponding solutions with respect to normal Tyrode containing isoprenaline is: $^{4}P < 0{\cdot}05$; $^{5}P < 0{\cdot}02$; $^{6}P < 0{\cdot}01$; $^{7}P < 0{\cdot}001$. The significance of the corresponding solution with respect to normal Tyrode containing adrenaline is: $^{8}P < 0{\cdot}02$; $^{9}P < 0{\cdot}001$.

The dilator effect of isoprenaline on the nictitating membrane, contracted by amphetamine, was not antagonized by chlorpropamide or AH 6848 (Table 3).

Stimulation of cardiac frequency and contractile force was blocked by propranolol and dichloroisoprenaline but not by chlorpropamide or carbutamide (Table 4).

Chlorpropamide (20 mg/kg) and dichloroisoprenaline (7 mg/kg) have themselves no effect on the blood glucose level. On simultaneous administration, however, there was definite decrease of the blood sugar in the first and second hours (Fig. 1).

Table 3. *The effect of chlorpropamide, AH 6848, dichloroisoprenaline and propranolol on the (—)-isoprenaline induced relaxation of the nictitating membrane of the cat contracted by amphetamine* (5 mg/kg., i.v.)

		% inhibition of relaxation induced by (—)-isoprenaline
(—)-Isoprenaline, 30 μg/kg		0
Chlorpropamide, 50 mg/kg	(4)	5 $*P < 0.05$
AH 6848, 50 mg/kg	(3)	7 $*P < 0.02$
Dichloroisoprenaline, 4 mg/kg	(8)	93 $*P < 0.01$
Propranolol, 0·7 mg/kg	(5)	94 $*P < 0.001$

Numbers in brackets indicate the number of experiments.
*Significance of the corresponding compound with respect to (—)-isoprenaline.

Table 4. *The effect of hypoglycaemic sulphonylureas on the (—)-isoprenaline induced changes of frequency and increase of isotonic muscular contraction of the heart*

		Changes in frequency (beats/min)	Increase of muscular contraction (mm)
(—)-Isoprenaline, 2.85×10^{-8} M	(12)	49 ± 12	19 ± 4
Carbutamide, 3.72×10^{-3} M and (—)-isoprenaline	(12)	45 ± 11	21 ± 5
(—)-Isoprenaline, 2.85×10^{-8} M	(12)	42 ± 3	19 ± 2
Chlorpropamide, 3.72×10^{-3} M and (—)-isoprenaline	(12)	38 ± 3	17 ± 1

Numbers in brackets indicate numbers of experiments. Recording of alterations of frequency was made only in half of these cases. There is no significant difference among the groups.

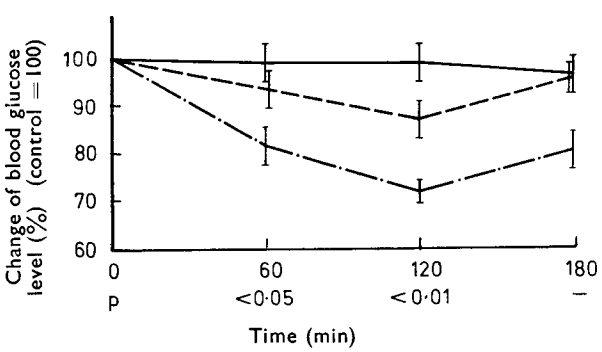

FIG. 1. The effect of dichloroisoprenaline and chlorpropamide on the blood glucose level of the intact rat. Each curve represents the mean values of ten rats. Vertical bars represent the standard error of mean. *P* represents the significance between chlorpropamide and chlorpropamide + dichloroisoprenaline. Dichloroisoprenaline (7 mg/kg, i.p.) ——. Chlorpropamide (20 mg/kg, i.p.) – – –. Dichloroisoprenaline (7 mg/kg) + chlopropamide (20 mg/kg) —·—·.

DISCUSSION

The results of the present studies demonstrate that two sulphonylureas, with hypoglycaemic activity, exert an antagonistic action against liver glycogenolysis and glucose release stimulated by β-adrenergic agonists. This property was also exhibited by insulin and propranolol. Another sulphonylurea without hypoglycaemic activity was not effective in this context. In our experiments, we were unable to demonstrate any β-blocking properties of the hypoglycaemic sulphonylureas either by actions on the nictitating membrane or hearts of cats. The effectiveness of propranolol confirms the results we obtained earlier with dichloroisoprenaline and pronethalol (Vizi & others, 1965) in suggesting that the response to isoprenaline is mediated by β-receptors. It may be argued that the mode of action of these β-receptor antagonists is specific β-blockade, although they also possess direct actions on cell membranes at concentrations similar to those used in this study (Lucchesi & Whitsitt, 1969). It also may be assumed that the local anaesthetic effect of the β-receptor blocking agents have a role in the phenomenon observed. Therefore we studied the effect of practalol [4-(2-hydroxy-3-isopropylaminopropoxy) acetanilide; ICI 50 172) which is devoid of local anaesthetic effect (Fitzgerald, 1969). Practalol, $4{\cdot}04 \times 10^{-4}$ M, failed to influence the glucose release augmented by glucose-free Tyrode solution or by isoprenaline ($5{\cdot}7 \times 10^{-8}$) neither was the decrease of glycogen level affected. But these observations did not throw any further light on the mechanism of the β-blocking agents studied because practalol is a very weak antagonist of isoprenaline (Dunlop & Schanks, 1968).

Failure to demonstrate peripheral β-blocking actions with sulphonylureas suggests that their mode of action in the liver differs from that of the β-blockers, in preventing catecholamine induced glycogenolysis.

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