J. Pharm. Pharmacol. 1979, 31: 598–600 Received November 20, 1978

Potentiation of the diabetogenic effect of streptozocin by phentolamine in the rat†

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The effect of α- and β-adrenoceptor blocking agents and a β_2-agonist, salbutamol, on the diabetogenic effects of streptozocin (threshold doses) was investigated in the rat. Phentolamine and salbutamol potentiated the diabetogenic effect of streptozocin but phenoxybenzamine, tolazoline, oxprenolol and propranolol were without effect. The potentiating effect of phentolamine was blocked by oxprenolol. Potentiation of the diabetogenic effect by phentolamine is not related to α-adrenoceptor block, vasodilatation or insulin release. But it may be related to its ability to stimulate β-adrenoceptors.

We have recently reported that the sympathetic nervous system does not appear to play any significant role in streptozocin-induced hyperglycaemia and tolbutamide and phenformin-induced hypoglycaemia in the rat (Kaul et al 1976). The evidence for this was obtained from experiments where drugs affecting sympathetic function were used. These drugs were 6-hydroxydopamine (6-OHD) and the α- and β-adrenoceptor blocking drugs phentolamine, phenoxybenzamine, propranolol and oxprenolol.

Iwatsuka et al (1974) reported that phentolamine pretreatment decreases the resistance of KK mice to the diabetogenic action of streptozocin. We have investigated the effect of various α- and β-adrenoceptor blocking drugs on the diabetogenic effects of threshold doses of streptozocin. This is because we reported earlier (Kaul et al 1976) that pretreatment with α- or β-adrenoceptor blocking drugs does not modify the severe hyperglycaemic response following 50 mg kg^{-1} i.v. of streptozocin.

METHODS

Female rats, 180–200 g, fasted for 18 h, were given different doses of streptozocin in the tail vein to find the threshold dose which was just ineffective in producing hyperglycaemia when blood sugar was measured 7 days after injections.

All drugs except streptozocin were dissolved in 0·9% NaCl and dosages and routes of administration are as shown in the Tables. Streptozocin was dissolved in citrate buffer pH 4·2 just before injection. Blood sugar measurements were carried out by an Auto Analyzer using Hoffman's micro method.

Plasma IRI was determined using the double antibody radio immunoassay procedure of Hales & Randle (1963) as modified by the Radiochemical Centre, Amersham. Statistical significance of blood sugar values was determined using Student's *t*-test. The number of animals showing hyperglycaemia in each group was analysed using Fischer's exact probability non-parametric test.

RESULTS

The effect of various doses of streptozocin on the blood sugar response is shown in Table 1. Doses of 15–25 mg kg^{-1} produced hyperglycaemia in only 0–10% of animals in most cases (hyperglycaemia was defined as blood sugar concentrations greater than 200 mg/100 ml). Higher doses of streptozocin

Table 1. Effect of phentolamine on the diabetogenic effect of streptozocin in the rats. All the values are means ± s.e.m. Figures in parentheses indicate the number of observations.

Treatment and dose mg kg^{-1}						
Phentolamine i.p.	Streptozocin i.v.	Blood sugar mg/100 ml Day 7		*P*	No rats with hyperglycaemia Day 7	%
—	15	134 ± 1·6	(20)		0/20	0
10	15	150 ± 17·2	(10)		2/10	20
20	15	151 ± 16·3	(10)		1/10	10
30	15	124 ± 1·3	(10)		0/10	0
—	20	169 ± 19·7	(10)I	I–II	1/10	10
10	20	210 ± 34·7	(9)	<0·01	3/9	33
20	20	292 ± 35·0	(9)II	I–III	6/9*	67
30	20	278 ± 44·8	(8)III	<0·05	5/8*	63
—	25	152 ± 2·19	(10)		0/10	0
10	25	204 ± 28·0	(9)		3/9	33
—	25	153 ± 1·7	(32)		0/32	0
20	25	262 ± 18·9	(30)	<0·001	18/30	60
—	25	173 ± 19	(10)		1/10	10
30	25	394 ± 25	(8)	<0·001	8/8	100
—	35	258 ± 26	(15)		11/15	73
—	40	279 ± 33	(15)		11/15	73
—	25	135 ± 2·0	(10)			
30†	25	132 ± 2·8	(10)			

* Phentolamine injected 15 min before streptozocin.
† Phentolamine injected 1 h after streptozocin.

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† Contribution No. 480 from CIBA-GEIGY Research Centre.

(35–40 mg kg^{-1}) produced hyperglycaemia in 73% of animals. Therefore, 25 mg kg^{-1} was selected as a threshold dose for studying the effects of various drugs.

Pretreatment of rats with phentolamine produced a dose-dependent significant increase in the blood sugar values ($P < 0.05$–0.001) and in the number of animals that became hyperglycaemic by day 7 ($P < 0.05$–0.001) (Table 1). This potentiating effect was observed only with streptozocin doses of 20 or 25 mg kg^{-1}. When phentolamine was administered 1 h after streptozocin, there was no potentiating effect (Table 1). Pretreatment with two other α-adrenoceptor blocking drugs, tolazoline (25 mg kg^{-1} i.p.) or phenoxybenzamine (5 mg kg^{-1} i.p.) had no significant potentiating effect on the streptozocin induced increase in blood sugar. Pretreatment with β-adrenoceptor blocking drugs, oxprenolol or propranolol, had no significant potentiating effect, rather both the drugs decreased blood sugar concentrations significantly when compared with controls ($P < 0.05$–0.01) (Table 2). Although β-adrenoceptor blocking drugs did not potentiate the diabetogenic action of streptozocin, oxprenolol pretreatment significantly antagonized the potentiating effect of phentolamine (Table 3). The number of rats showing hyperglycaemia was also reduced significantly by this pretreatment. Since oxprenolol blocked the potentiating effect of phentolamine, we investigated the effect of salbutamol, a β_2-receptor agonist on the diabetogenic properties of streptozocin. At 3 mg kg^{-1} i.p., salbutamol potentiated slightly the diabetogenic properties of streptozocin and the number of animals which became hyperglycaemic was significantly higher than control ($P < 0.05$).

Table 2. Effect of oxprenolol and propranolol on the diabetogenic effect of streptozocin in the rat. All values are the mean ± s.e.m. Figures in parentheses indicate the number of observations.

Treatment and dose mg kg^{-1}	Blood sugar mg/100 ml (Day 7)		No rats with hyperglycaemia (Day 7)	
Streptozocin 25 i.v.	177 ± 16·1	(10)	3/10	(30%)
Oxprenolol 10 i.p. + Streptozocin 25 i.v.	149 ± 12·1	(10)	1/10	(10%)
Oxprenolol 20 i.p. + Streptozocin 25 i.v.	128 ± 2·2**	(10)	0/10	(0%)
Propranolol 20 i.p. + Streptozocin 25 i.v.	131 ± 2·9*	(10)	0/10	(0%)

* $P < 0.05$. ** $P < 0.01$.

Phentolamine has been reported to increase insulin secretion in the rat (Furman & Tayo 1974). We have also observed a marked increase in insulin secretion (+124% at 30 min) following phentolamine at a dose of 20 mg kg^{-1} i.p. The insulin values (mean ± s.e.) in μU ml^{-1} in the normal and phentolamine-treated animals were 40·9 ± 1·87 and 91·8 ± 22·7 respectively. Pretreatment of rats with insulin 0·5 unit 15 min or 1 unit s.c. 60 min before, or sodium tolbutamide 100 mg kg^{-1} p.o. 1 or 2 h before, did not potentiate the diabetogenic properties of streptozocin (25 mg kg^{-1}).

Table 3. Effect of oxprenolol and oxprenolol + phentolamine on the diabetogenic effect of streptozocin in the rat. All values are the mean ± s.e.m. Figures in parentheses indicate the number of observations. Oxprenolol was injected 45 min before streptozocin. Phentolamine was injected 15 min before streptozocin.

Treatment and dose mg kg^{-1}					No rats with hyperglycaemia	
Oxprenolol s.c.	Phentolamine i.p.	Streptozocin i.v.	Blood sugar mg/100 ml		Day 7	%
—	25	—	157 ± 1·88 (20)	I	0/20	0
—	25	20	306·7 ± 23·63 (17)	II	13/17	76
20	25	20	189·6 ± 18·08 (20)	III	5/20	25*
20	25	—	152·1 ± 3·27 (20)		0/20	0

I–II $P < 0.001$
II–III $P < 0.001$
* $P < 0.05$

DISCUSSION

The results show that phentolamine (10–30 mg kg^{-1}), an α-adrenoceptor blocking agent, potentiates the diabetogenic effect of streptozocin (20–25 mg kg^{-1}) in a dose-dependent manner in the rat. These results are in general agreement with those of Iwatsuka et al (1974) in the KK mouse. As far as we know this is the first study in the rat where a pharmacological potentiation of streptozocin has been demonstrated. Most earlier studies have dealt with inhibition of the diabetogenic response to streptozocin (Dulin & Wyse 1969; Lazarus & Shapiro 1973; Ganada et al 1976). The potentiation of the diabetogenic property of streptozocin by phentolamine could be due to several factors. Phenoxybenzamine and tolazoline produced significant α-adrenoceptor block at the dosages used. Phenoxybenzamine, which is an even more potent α-adrenoceptor blocking drug than phentolamine, had no significant potentiating effect even when given 4 h before streptozocin. This suggests that potentiation of the diabetogenic property of streptozocin by phentolamine is not related to its α-adrenoceptor blocking action.

We have confirmed the findings that phentolamine stimulates insulin secretion in the rat (Furman & Tayo 1974). The potentiating effect cannot be

ascribed to insulin release, since insulin or tolbutamide (a known stimulator of insulin secretion) did not modify the streptozocin response. Nor can the potentiating effect of phentolamine be due to its vasodilatatory effect since phenoxybenzamine, a marked vasodilator, did not show this effect. Phentolamine has been reported to stimulate insulin secretion as a result of unopposed endogenous β-adrenoceptor stimulation (Robertson & Porte 1973; Woods & Porte 1974). Our results with oxprenolol suggest that the potentiating effect of phentolamine could be due to it having a β-adrenoceptor stimulating property. This was further supported by using a β_2-agonist salbutamol when the number of animals that became hyperglycaemic was significantly higher compared with controls.

It is also possible that phentolamine in some way alters the permeability of β-cells to streptozocin and thereby potentiating the diabetogenic effect. This property cannot be attributed to membrane stabilization since propranolol, a known membrane stabilizing drug, did not show any potentiating effect (Langslet 1970).

Acknowledgements

The authors are grateful to Dr Sheela Talwalker for the help in statistical analysis and Mr N. D. Desai, Mr N. N. Shah and Mrs R. Srivastava for expert technical assistance.

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