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Influence of inhibition of extraneuronal uptake and of *O*-methylation on the hyperglycaemia caused by sympathomimetic amines in depancreatized rats

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Abstract—This study aimed at testing whether the *O*-methylating system (extraneuronal uptake + *O*-methylation) modulates, in-vivo, β-adrenoceptor-mediated responses. The influences of U-0521 (3,4-dihydroxymethylpropiophenone, an inhibitor of catechol-*O*-methyl transferase (COMT)) and hydrocortisone (an inhibitor of extraneuronal uptake) on the hyperglycaemia evoked by isoprenaline and adrenaline were compared. Both inhibitors enhanced the increase of the plasma glucose level induced by either isoprenaline (0·36 nmol kg^{-1} min^{-1}) or adrenaline (0·55 nmol kg^{-1} min^{-1}). The enhancement caused by U-0521 developed faster than that caused by hydrocortisone, but was of the same magnitude. This is the first report of supersensitivity to sympathomimetic amines caused by inhibition of either COMT or extraneuronal uptake in-vivo and for a response not involving smooth muscle cells.

Extraneuronal uptake for a sympathomimetic amine is able to generate a concentration gradient from the incubation medium to the extracellular space. Inhibition of extraneuronal uptake thus increases the concentration of this amine in the biophase and supersensitivity to it ensues (Trendelenburg 1972, 1988; Guimarães 1982).

The degree of supersensitivity caused by inhibition of extraneuronal uptake indicates its importance in modulating the response to agonists which are its substrates (Furchgott 1972; Trendelenburg 1972; Belfrage 1978; Guimarães 1982).

As shown by several authors, reuptake into the nerve is very important in modulating responses mediated by α-adrenoceptors (Trendelenburg 1966; Langer & Trendelenburg 1969; Guimarães et al 1971; Rosell & Belfrage 1975; Guimarães & Paiva 1981a, b) while extraneuronal uptake and *O*-methylation is very important in modulating responses mediated by β-adrenoceptors (Guimarães 1975; Guimarães et al 1975; Guimarães & Paiva 1977a, b).

It has been suggested that the β_1-adrenoceptor responds primarily to released neurotransmitter, whereas the β_2-adrenoceptor is responsive to circulating catecholamines (Ariëns & Simonis 1976; Bryan et al 1981; Broadley et al 1984; Broadley 1990).

Until now, the capability of extraneuronal *O*-methylating systems in modulating β-adrenoceptor-mediated responses has been shown for smooth muscle cell responses only (Guimarães et al 1978; Zaagsma et al 1979; Broadley et al 1984; Proença et al 1988, 1990).

The present study was undertaken to compare the influence of inhibition of extraneuronal uptake and *O*-methylation on hyperglycaemia induced by sympathomimetic amines, an effect which is supposed to be primarily dependent on β_2-adrenoceptor stimulation (Lefkowitz et al 1990).

Materials and methods

Materials. Drugs used were: (−)-adrenaline bitartrate (Sigma, St Louis, USA); cocaine hydrochloride (Uquipa, Lisboa, Portugal); hydrocortisone hemisuccinate-21 sodium salt (Sigma); (−)-isoprenaline (Sigma); phentolamine hydrochloride (Ciba Geigy, Lisboa, Portugal); U-0521 (3,4-dihydroxymethylpropiophenone (Upjohn, Kalamazoo, USA). Stock solutions of isoprenaline were prepared in 0·9% NaCl (saline) containing approximately 0·01 M HCl. For other drugs stock solutions were prepared in saline. All solutions were kept at 4°C when not in use. When needed, final solutions of drugs were made in medium immediately before use.

Methods. Male Wistar rats (Fundação Gulbenkian, Portugal), 250–350 g, were maintained on a 12 h light/12 h dark cycle, and were fed a standard pellet diet; tap water was freely available. Food pellets were withdrawn 12 h before experimentation. After being anaesthetized with pentobarbitone (50 mg kg^{-1}), the rats were pancreatectomized and each animal had two cannulae implanted, one into the left carotid artery for collection of blood samples and another into the right femoral vein for the infusion of drugs or saline.

Immediately after cannulation of the vessels, 5·9 μmol kg^{-1} cocaine (to inhibit re-uptake into the nerve) and 5·5 μmol kg^{-1} phentolamine (to block α-adrenoceptors) were injected intravenously.

Fifteen min after these injections (time zero), a control blood sample of 0·2 mL was collected and the rats were given saline or U-0521 (a catechol-*O*-methyl transferase (COMT) inhibitor) or hydrocortisone (an inhibitor of extraneuronal uptake) by intravenous infusion. The infusion of saline or of the inhibitor was made by a peristaltic pump (Gilson Minipuls 2) providing a flux of 0·03 mL min^{-1}. Hydrocortisone caused hyperglycaemia by itself which became significant only from 35 min onwards. Thus, the infusion of both hydrocortisone and U-0521 was stopped at 30 min, at which time a second sample was collected and the infusion of the sympathomimetic amine started. From then onwards, a blood sample was collected every 5 min for 35 min so that seven samples in total were collected per experiment. The amine infusion lasted 25 min.

Biochemical determinations. Plasma glucose was determined by the glucose oxidase-PAP (phenol-4-amino-phenazone) method (Boehringer-Mannheim, Germany).

Calculations and statistics. The values represent the arithmetic means ± s.e.m. of the indicated number of rats in separate experiments. Significance was evaluated by analysis of variance (Wallenstein et al 1980). A bilateral probability level of 0·05 or less was considered statistically significant.

Results

Hyperglycaemic response to isoprenaline and adrenaline. Since inhibition of either *O*-methylation or extraneuronal uptake was supposed to enhance the hyperglycaemic action of isoprenaline and adrenaline, the smallest effective dose of either amine was determined in preliminary experiments (0·36 nmol kg^{-1} min^{-1} for isoprenaline and 0·55 nmol kg^{-1} min^{-1} for adrenaline) and used in this study.

Intravenous infusion of either isoprenaline or adrenaline

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caused a gradual hyperglycaemic response which became significant only 10 min after starting the infusion and was still increasing at the moment of stopping the infusion (at 25 min).

Influence of U-0521. By itself, U-0521 had no effect on basal glucose levels. However, it significantly enhanced the hyperglycaemic response to either isoprenaline or adrenaline (Tables 1, 2). The supersensitivity caused by U-0521 developed faster for adrenaline (it appeared at 5 min) than for isoprenaline (10 min).

Influence of hydrocortisone. Preliminary experiments showed that hydrocortisone by itself causes a hyperglycaemic response which became significant only from 35 min onwards (not shown). Before this moment had been reached, hydrocortisone clearly enhanced the hyperglycaemic response caused by both isoprenaline and adrenaline (Tables 1, 2).

As for U-0521, the supersensitivity caused by hydrocortisone appeared earlier with adrenaline (at 100 min) than with isoprenaline (at 15 min).

Discussion

The present results show that both isoprenaline and adrenaline cause hyperglycaemia in pancreatectomized rats pretreated with phentolamine and cocaine. The predominant population of adrenoceptors mediating the hyperglycaemia in the rat seems to belong to the β-type (Lefkowitz et al 1990) as 0·36 nmol L^{-1} isoprenaline and 0·55 nmol L^{-1} adrenaline caused equivalent effects.

Both inhibition of extraneuronal uptake by hydrocortisone and inhibition of COMT by U-0521 enhance the hyperglycaemia induced by either isoprenaline or adrenaline. This enhancement of the amine-induced hyperglycaemia by either inhibitor is expressed by a displacement of the dose-response curve for the amines to the left and provides good evidence for the view that, in-vivo, the extraneuronal *O*-methylating system functions as a site of loss. This is the first report of supersensitivity to sympathomimetic amines caused by inhibition of the extraneuronal *O*-methylating system in-vivo not involving smooth muscle cells.

Although the overall influence of U-0521 is very similar to that of hydrocortisone, there are some differences between the influence exerted by U-0521 and by hydrocortisone on the effect caused by either amine, and between the influence exerted by either inhibitor on the effects of the two amines. Firstly, the enhancement by U-0521 of isoprenaline-induced hyperglycaemia became apparent earlier than that caused by hydrocortisone. At 10 min, the hyperglycemia caused by isoprenaline in the rat pre-infused with U-0521 was already significantly higher than that caused in the controls, while, only at 15 min, was the hyperglycaemia caused by isoprenaline higher in the animals pre-infused with hydrocortisone than in the controls. As for isoprenaline, the supersensitivity to adrenaline appears earlier in the animals pre-infused with U-0521 than in those pre-infused with hydrocortisone; at 5 min, the enhancement by U-0521 of the adrenaline-induced hyperglycaemia was already significant, while that caused by hydrocortisone became significant only at 10 min.

The present results provide no answer to the question: from where comes the enhancing effect? Most probably the liver plays a major role in the development of such a supersensitivity, as it is the site of glucose metabolism and possesses high COMT activity (Guldberg & Marsden 1975). However, other tissues such as the blood vessels which contain high COMT activity may play a role (Guldberg & Marsden 1975).

In the majority of tissues where the comparison has been made, the responses to sympathomimetic amines are more enhanced by the inhibition of COMT than by the inhibition of extraneuronal uptake (Kaumann 1972; Proença et al 1988). This is explained by the fact that in most tissues there are hydrocorti-

Table 1. Effect of isoprenaline (0·36 nmol kg^{-1} min^{-1}) on the plasma glucose level of the rat (mmol L^{-1}) and the influence of hydrocortisone (20·7 μmol kg^{-1}) and of U-0521 (41·5 μmol kg^{-1}) on the effect of isoprenaline. The basal glucose levels (t_0) and the variations from control values are shown. The animals were pancreatectomized and pretreated with phentolamine (5·5 μmol kg^{-1}) and cocaine (5·9 μmol kg^{-1}). Values given are means ± s.e.m.

	Increase in blood glucose (time (min) after starting the infusion)					
	0	5	10	15	20	25
Infusion						
Saline (control)	6·22 ± 0·15	0·12 ± 0·03	0·23 ± 0·03	0·27 ± 0·02	0·31 ± 0·03	0·35 ± 0·04
Isoprenaline	6·44 ± 0·16	0·27 ± 0·04	0·72 ± 0·06	1·3 ± 0·07	1·65 ± 0·13	2·18 ± 0·19
Isoprenaline (after hydrocortisone)	6·70 ± 0·18	0·36 ± 0·06	0·83 ± 0·10	1·87 ± 0·10*	2·29 ± 0·24*	2·90 ± 0·26*
Isoprenaline (after U-0521)	6·50 ± 0·12	0·54 ± 0·06	1·58 ± 0·08*	1·93 ± 0·08*	2·47 ± 0·12*	3·23 ± 0·18*

* Significant differences between these values and those obtained with isoprenaline alone. The differences between isoprenaline and saline controls from 10 min onwards were significant.

Table 2. Effect of adrenaline (0·55 nmol kg^{-1} min^{-1}) on the plasma glucose level of the rat (mmol L^{-1}) and the influence of hydrocortisone (20·7 μmol kg^{-1}) and of U-0521 (41·5 μmol kg^{-1}) on the effect of adrenaline. The basal glucose levels (t_0) and the variations from control values are shown. The animals were pancreatectomized and pretreated with phentolamine (5·5 μmol kg^{-1}) and cocaine (5·9 μmol kg^{-1}). Values given are means ± s.e.m.

	Increase in blood glucose (time (min) after starting the infusion)					
	0	5	10	15	20	25
Infusion						
Saline (control)	6·00 ± 0·13	0·15 ± 0·04	0·22 ± 0·02	0·39 ± 0·02	0·43 ± 0·05	0·55 ± 0·05
Adrenaline	6·27 ± 0·15	0·24 ± 0·05	0·66 ± 0·03	1·08 ± 0·09	1·60 ± 0·12	2·22 ± 0·20
Adrenaline (after hydrocortisone)	6·50 ± 0·22	0·45 ± 0·06	1·15 ± 0·12*	1·90 ± 0·22*	2·56 ± 0·30*	2·98 ± 0·41*
Adrenaline (after U-0521)	6·78 ± 0·19	0·95 ± 0·07*	1·49 ± 0·10*	2·00 ± 0·18*	2·56 ± 0·22*	2·84 ± 0·15*

* Significant differences between these values and those obtained with adrenaline alone. The differences between adrenaline and saline controls from 10 min onwards were significant.

sone-resistant *O*-methylating sites, while there is practically no U-0521-resistant COMT (Graefe & Trendelenburg 1974; Azevedo & Osswald 1976; Garland et al 1981; Broadley & Paton 1990; Proença et al 1990). In the present experiments the maximum supersensitivity caused by U-0521 was of the same magnitude as that caused by hydrocortisone, showing that hydrocortisone is a good inhibitor for extraneuronal uptake at the sites modulating this metabolic response to the catecholamines.

Finally we should like to consider the fact that the enhancement of the effect of both isoprenaline and adrenaline by U-0521 develops faster than that caused by hydrocortisone. This seems to indicate that there is a certain amount of extracellularly facing plasma membrane-bound COMT (Head et al 1985). If all the COMT were intracellularly located, the amine would have to be taken up into intracellular sites, to be accumulated and to be transported outward in such a way that the supersensitivity induced by U-0521 would most probably occur as a secondary sensitization (Furchgott & Sanchez Garcia 1968) and much later than that caused by hydrocortisone.

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