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Action of dopamine and noradrenaline on synaptic transmission in sympathetic ganglia of brown fat

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BROWN adipose tissue has a dual sympathetic nerve supply.[1,2] There are postganglionic fibres of neurones in the paravertebral ganglia, supplying blood vessels and mediating vasodilatation. Another set arise from ganglia embedded within the adipose tissue. They supply the adipocytes, mediating lipolysis, stimulation of respiration and thermogenesis. This latter system has an easily measurable action on target tissue (stimulation of respiration) and offers a useful model for the study of synaptic transmission. In this communication it will be shown that dopamine produces sustained facilitation of synaptic transmission across the cholinergic synapse. This may be due to increased prostaglandin E_2 synthesis in the postsynaptic neurone. It is also suggested that noradrenaline may inhibit synaptic transmission by enhancement of prostaglandin E_3 formation in ganglion cells.

Methods

Interscapular brown fat was taken from 21-day-old rats maintained at animal house temperature (22°). Respiration rate was measured on individual lobules suspended in Ringer–Locke solution in a well-type oxygen electrode, maintained at 25°. Various drugs were added to the well after the establishment of a basal oxygen consumption rate as shown in Fig. 1.

The rate of prostaglandin synthesis in paravertebral ganglia was determined by the method of Van Dorp *et al.*[3] Rat paravertebral ganglia (0·1 g) were homogenized with a glass hand homogenizer in 1 ml of ice-cold solution containing 130 mM KCl, 20 mM tris buffer pH 7·4 and 5 mM $MgCl_2$. Homogenate (0·1 ml) was added to 1 ml of solution containing 1 mM (50,000 counts/min) of either 1-C 14 labelled arachidonic acid (PGE_2 precursor) or 1-C 14 tagged eicosapentaenoic acid (PGE_3 precursor) together with 0·3 mM reduced glutathione, 0·3 mM hydroquinone, 100 mM tris buffer pH 7·4 and 5 mM $MgCl_2$. After incubation for 1 hr at 37° the reaction was stopped by adding 1 ml of 0·2 M citric acid. Prostaglandins were extracted with two aliquots of ether. The ether extract was resolved on silica thin layer chromatoplates developed in benzene–dioxan–acetic acid (20:20:1, by vol.). Areas corresponding to a carrier prostaglandin E_1 were scraped for radioactivity measurement by liquid scintillation counting. 1-C 14 labelled arachidonic acid and eicosapentaenoic acid

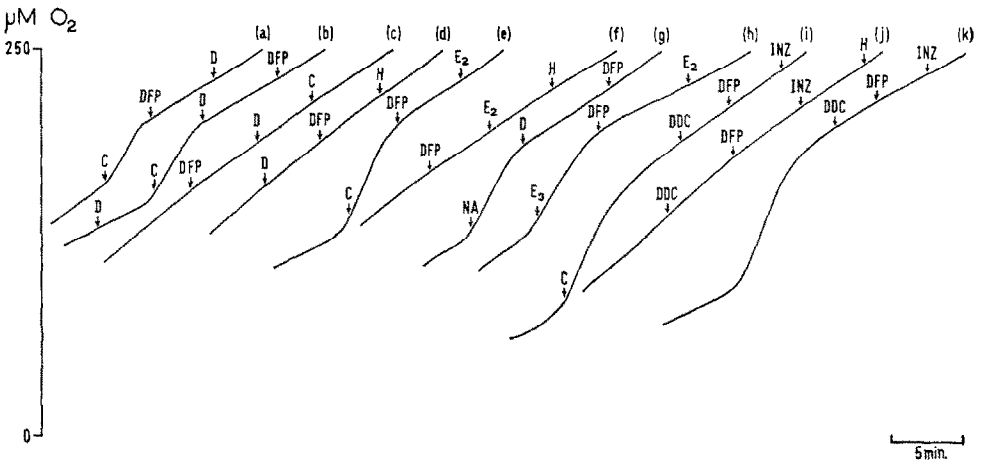

FIG. 1. A typical experiment showing the effect of various drugs on the respiration of brown fat lobules. D = Dopamine 10^{-7} M. DFP = Diisopropyl fluorophosphate 10^{-8} M. C = Tubocurarine 10^{-8} M. H = Hemicholinium 10^{-8} M. E_2 = Prostaglandin E_2 10^{-7} M. E_3 = Prostaglandin E_3 10^{-7} M. NA = Noradrenaline 10^{-7} M. INZ = Iproniazide 10^{-7} M. DDC = Diethyldithiocarbamate 10^{-6} M.

were isolated from Euglena gracilis Z grown in the dark on a synthetic medium containing 1-C 14 linoleic acid. The procedure used was exactly as described in Ref. 4.

Results and discussion

Lipolysis and increased respiration in brown fat adipocytes induced by sympathetic stimulation or catecholamines are mediated via typical β receptors. It was predictable therefore that dopamine (10^{-7} M) would have had no effect on basal respiration (Fig. 1a). Acetylcholine esterase inhibitors such as diisopropyl fluoro phosphate (DFP), though they increase acetyl choline at the synapse, cannot build up a threshold level of the transmitter to stimulate autonomic ganglia. However, both drugs together caused marked stimulation of respiration of brown fat lobules (Fig. 1a and b). The enhanced respiration was blocked by tubocurarine (10^{-8} M) added before the other drugs or after the establishment of fast respiration (Fig. 1a, b and c). After the addition of tubocurarine, a 10-fold increase in dopamine did not restore enhanced oxygen consumption. Hemicholinium (10^{-8} M) which prevents presynaptic release of acetylcholine, prevented the action of dopamine + DFP (Fig. 1d). These observations suggests that dopamine causes subthreshold levels of acetylcholine to be effective in firing the postsynaptic neurone.

The increased respiration produced by DFP + dopamine persisted after rinsing the tissue with several aliquots of 30 ml of drug free Ringer–Locke solution. (This suggests that dopamine produces its effect through a long lasting metabolic action.)

Table 1 shows that dopamine stimulated PGE_2 synthesis in paravertebral ganglia. It can be seen from Fig. 1 (e and f) that PGE_2, though by itself did not change basal respiration, it cooperated with DFP enhancing oxygen consumption; their combined action was blocked by tubocurarine and prevented by prior incubation with hemicholinium. It could be that PGE_2, or some action of it (e.g. stimulation of adenylate kinase[5]), is responsible for the sustained facilitation of synaptic transmission by dopamine.

A small dose of noradrenaline (10^{-7}) inhibited dopamine-stimulated respiration (Fig. 1g). Noradrenaline could have the opposite effect of dopamine on synaptic transmission. It stimulated PGE_3 formation in paraventebral ganglia (Table 1). PGE_3 reverses PGE_2-stimulated adenylate kinase.[5] It also reversed dopamine and PGE_2 enhanced respiration in brown fat (Fig. 1h).

Diethyl dithiocarbamate inhibits dopamine hydroxylase by copper chelation.[6] At a concentration of 10^{-6} M together with a monoamine oxidase inhibitor (iproniazid 10^{-7} M) and DFP it caused gradual stimulation of respiration (Fig. 1,i and j). This is explained as due to partial replacement of noradrenaline, spontaneously released at postganglionic endings, with dopamine. The latter may leak to the synaptic neurones facilitating their stimulation. Fast respiration could be checked by tubocurarine and prevented by prior incubation of the tissue with hemicholinium. A high dose of diethyl dithiocarbamate (10^{-5} M) caused stimulation followed by resumption of basal respiration, presumably after total blockage of noradrenaline synthesis (Fig. 1, k).

TABLE 1. THE ACTION OF DOPAMINE AND NORADRENALINE ON THE BIOSYNTHESIS OF PGE_2 AND PGE_3 IN HOMOGENATES OF PARAVERTEBRAL GANGLIA

	Basal	Dopamine	Dopamine + Phentol-amine	Dopamine + Propronolol	Noradren-aline	Noradren-aline + Phentol-	Noradren-aline + Propro-nolol
PGE_2	4·1	9·8	3·9	9·6	3·8	3·9	3·8
PGE_3	3·6	3·7	3·4	3·6	8·0	7·7	3·5

The results (means of five experiments) as expressed as nanomoles of prostaglandin synthesized per 10 mg protein per hour. The drugs were added at a concentration of 10^{-7} M.

In a thoroughly teased preparation of brown fat, or free fat cells prepared by collagenase[7] dopamine and diethyl dithiocarbamate + iproniazid, alone or together with DFP had no action on basal respiration. This is presumably due to disruption and loss of ganglia. In such preparations, as is well known, noradrenaline stimulated respiration by acting directly on the β-receptors of adipocytes.

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The effect of (+)-catechin on the hepatic level of ATP and the lipid content of liver during experimental steatosis

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THE BIOCHEMICAL effect of the bioflavonoids is unknown. The common name "vitamin P", often used to designate them, has been strongly criticized. Many authors think that several reported physiological actions of the flavonoids could be ascribed to the ascorbic acid content of the preparations used.

Our attention was drawn to a possible role of flavonoids in the biosynthesis of ATP by the observation of Teras.[1–3] This author has reported that purified preparations of rutin and catechins uncoupled oxidative phosphorylation and stimulated the activity of ATP-ase in rats and in mitochondria isolated from their livers.

In our experiments with a chemically pure preparation of (+)-catechin[4] we have found that the