TRIFLUOPERAZINE, AN INHIBITOR OF CALMODULIN ACTION, ANTAGONISES PHENYLEPHRINE-INDUCED METABOLIC RESPONSES AND MITOCHONDRIAL CALCIUM FLUXES IN LIVER

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1. Introduction

The stimulation of glycogenolysis and gluconeogenesis by α-adrenergic agonists in liver may be associated with a transient increase in the concentration of cytoplasmic Ca^{2+} (see [1]). Some uncertainty appears to exist as to the source of this Ca^{2+} (cf. [2–4] and [5,6]), although the ability of mitochondria of participate in the sequestration of this pulsed Ca^{2+} now seems established [7].

In view of the apparent involvement of calmodulin in numerous Ca^{2+}-dependent metabolic events [8–10], it seemed important to consider whether α-adrenergic agonist-induced changes in liver metabolism involve this protein. This study, using the perfused rat liver system, provides evidence that trifluoperazine, an agent used to assess the action of calmodulin [8,9,11], antagonises the α-adrenergic agonist-induced stimulation of glucose output, oxygen consumption and mitochondrial Ca^{2+} fluxes.

2. Methods

Livers from female Wistar strain albino rats (~200 g body wt) were perfused with Krebs-Henseleit bicarbonate medium equilibrated with 95% O_2–5% CO_2 essentially as in [12]. Minor modifications were the use of a non-recirculating medium at 32°C [13] containing 1.65 mM $CaCl_2$. The flow rate was maintained at 15 ml/100 g body wt. The effluent medium was monitored for oxygen content using a Rank oxygen electrode, and the flow rate through the liver determined by measuring the volume of the effluent collected in a fixed time. Glucose released by the liver was estimated using the glucose oxidase method (Sigma assay kit 510-A). 'Heavy' mitochondria [14] were isolated from separate lobes at 0–4°C. Each lobe was completely homogenised within 10 s of its removal from the intact organ. Measurement of mitochondrial Ca^{2+} influx, Ca^{2+} retention and the protonmotive force was as in [7,15,16]. Mitochondrial protein was determined as in [17]. All reagents were of analytical reagent grade. Hormones were purchased from the Sigma Chemical Co., St Louis, MO. Trifluoperazine was a generous gift of Smith, Kline and French.

3. Results

Fig.1. shows the effect of phenylephrine on glucose output and oxygen consumption by the livers of fed rats, pre-perfused for 10–15 min with or without trifluoperazine (3.5×10^{-6} M). Data in fig.1a indicate that the output of glucose from liver is enhanced immediately following the administration of phenylephrine (2×10^{-6} M). Such output is maximal by ~2 min and continues for the duration of the experiment. The data also show that pre-perfusion of the liver for 10 min with trifluoperazine reduces this response to phenylephrine by ~80%.

Oxygen consumption by the liver increases immediately following infusion of phenylephrine to reach maximal values by ~2 min and continues for at least 7 min (fig.1b). This response is diminished in magnitude by ~50% in the presence of trifluoperazine.

By contrast, glucagon administration induced slower responses in respect of both glucose output and oxygen consumption and these were unaffected

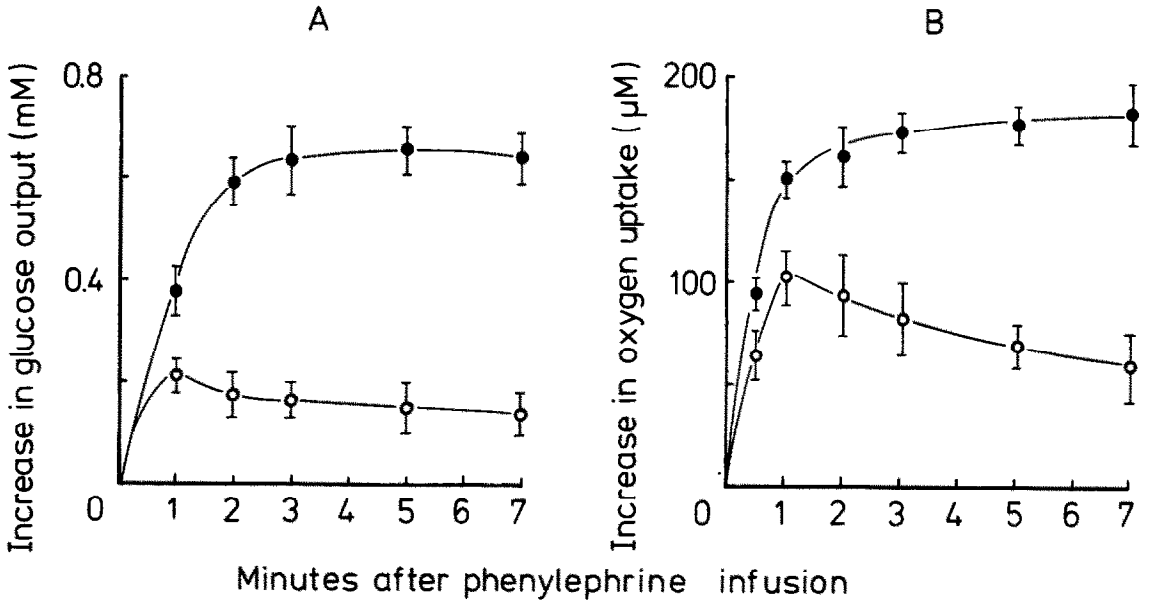

Fig.1. Effect of trifluoperazine on phenylephrine-induced glucose release and oxygen output in perfused rat liver. Livers of fed rats were perfused initially for 5 min with oxygenated Krebs-Henseleit buffer as in section 2. Trifluoperazine (3.5×10^{-6} M) was then infused where indicated and 15 min later, phenylephrine (2×10^{-6} M) was infused for a further 7 min. Glucose release (A) was estimated by collecting 600 μl samples of the effluent medium, removing any contaminating erythrocytes by centrifugation, and assaying portions (50–200 μl) of the supernatant. The amount of glucose release prior to phenylephrine infusion was 0.4–0.8 mM. Oxygen consumption (B) was estimated from the difference between influent and effluent oxygen concentrations. The amount of oxygen consumed prior to infusion of phenylephrine was 300–500 μM. Results shown are the mean of 5 separate expt. (○) Trifluoperazine infused; (●) trifluoperazine absent.

by pre-perfusion with the calmodulin inhibitor (not shown).

Fig.2a shows the mean of 5 individual expt. in which Ca^{2+} influx was determined in mitochondria isolated from the lobes of livers perfused with or without phenylephrine. The initial rate of Ca^{2+} transport is stimulated ~80% over that of the control (cf. [7]). Fig.2b shows that perfusion of the liver with trifluoperazine itself does not alter the control rate of Ca^{2+} influx but completely prevents the stimulation induced by the hormone. Similar effects of the α-agonist and trifluoperazine were observed on mitochondrial Ca^{2+} influx when the initial [Ca^{2+}] was reduced to 5 μM. Perfusion with glucagon did not alter initial rates of Ca^{2+} influx, and pretreatment of livers with trifluoperazine was without effect (not shown).

The time for which mitochondria were able to retain a fixed amount of Ca^{2+} [15] was also increased following pre-perfusion for 7 min with 2×10^{-6} M phenylephrine (3.5 ± 0.3 vs 6.9 ± 0.9 min, $n = 4$). Trifluoperazine 3.5×10^{-6} M) exerted effects similar to those described above for Ca^{2+} influx. The calmodulin inhibitor had no effect on Ca^{2+} retention alone but inhibited the effect of phenylephrine by 39 ± 14% ($n = 4$).

In some experiments the components of the protonmotive force, the mitochondrial transmembrane pH gradient and membrane potential, were also determined since these parameters are indicators of both the functional integrity of the organelle and under some conditions, of the capacity of the mitochondria to sequester and retain Ca^{2+} [7,18]. The transmembrane pH gradient and membrane potential of mitochondria prepared from livers perfused for 7 min with phenylephrine (2×10^{-6} M) (see fig.2), is increased by 27 ± 6% and 3 ± 0.5% ($n = 3$), respectively, when compared to their appropriate controls (control transmembrane pH gradient, 75.3 ± 1.7 mV ($n = 3$); membrane potential, 139.6 ± 2.8 mV ($n = 3$).

By contrast, pre-perfusion of livers with glucagon or trifluoperazine alone had no effect on the mitochondrial transmembrane pH gradient or the membrane potential in subsequently prepared mitochon-

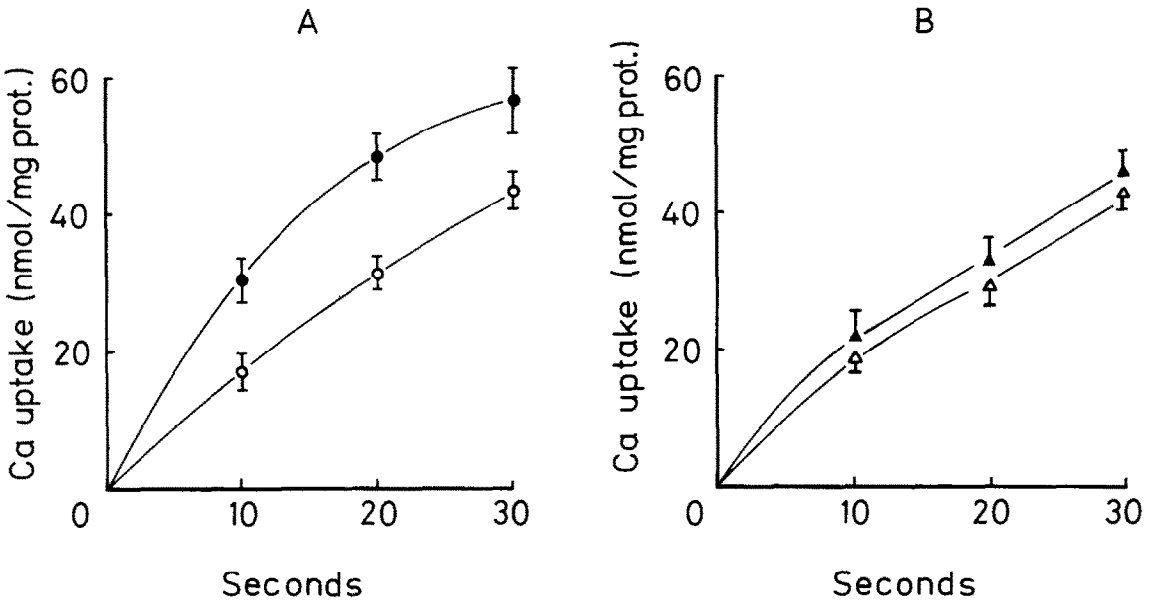

Fig.2. Effect of trifluoperazine on phenylephrine-induced stimulation of the initial rate of mitochondrial Ca^{2+} influx. Livers of fed rats were perfused as indicated in fig.1 and section 2. Prior to infusing phenylephrine, the median lobe was removed, homogenised, and the mitochondria isolated. The liver was allowed to recover for 3 min, and then phenylephrine was infused into the remaining lobes for 7 min. At this time the papilliform and left main lobes were removed, homogenised and the mitochondria isolated. The initial rate of mitochondrial Ca^{2+} transport was then measured [7] using 50 μM $CaCl_2$ containing 0.3 μCi $^{45}Ca^{2+}$. In (A) trifluoperazine was absent. In (B) trifluoperazine (3.3×10^{-6} M) was infused for 15 min prior to the infusion of phenylephrine. Results shown are the mean of 5 separate expt. (○,△) refer to mitochondria isolated from median lobes. (●,▲) mitochondria isolated from papilliform and left main lobes.

dria. Pre-perfusion of livers with trifluoperazine prior to administration of phenylephrine, however, reduced the stimulatory effect of the α-agonist on the transmembrane pH gradient by 52 ± 8% (n = 3).

4. Discussion

Phenothiazine antipsychotic agents such as trifluoperazine have been used in a number of studies to assess the potential involvement of calmodulin in intracellular Ca^{2+}-dependent reactions [8,11,19,20]. Experiments in this report establish that pre-perfusion of rat liver with trifluoperazine, antagonises the stimulation by phenylephrine of glucose output and oxygen consumption. The studies in [21] indicate that the bulk of this oxygen consumption is attributable to mitochondria, and other workers have observed that several mitochondrial energy-linked reactions are sensitive to prior hormone treatment of the intact tissue [22–25]. Here we show that trifluoperazine also antagonises the stimulation of the protonmotive force induced by phenylephrine. The data also confirm our findings that α-adrenergic agonists stimulate mitochondrial Ca^{2+} influx [7], an effect we show here to be antagonised by the calmodulin inhibitor.

On the other hand, trifluoperazine failed to antagonise glucagon-induced metabolic changes in the perfused liver. This, as well as its inability to alter the protonmotive force in the subsequently isolated mitochondria, provides support for a specific action of the inhibitor. The data also support the view that α-adrenergic agonists act through a mechanism different from that of glucagon [26,27].

Finally, if one assumes that trifluoperazine specifically inhibits the action of calmodulin, then some of the physiological responses of α-adrenergic agonists must involve calmodulin. Since one of these early responses is a redistribution of cytoplasmic Ca^{2+} [1–7,26,27], it is of some interest that trifluoperazine also antagonises phenylephrine-induced stimulation of mitochondrial Ca^{2+} transport. Thus calmodulin appears to be involved in the mechanism of action

of the α-adrenergic agonists, and may be a regulator of mitochondrial Ca^{2+} fluxes in liver. The interrelationships of these calmodulin-mediated events is currently under further investigation.

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