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Short communication

The effect of tolbutamide on cerebral blood flow during hypoxia and hypercapnia in the anaesthetized rat

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Abstract. The increase in blood flow in the cerebral cortex of the anaesthetized rat during hypoxia and hypercapnia was investigated. Cerebral blood flow (CBF) was measured using the hydrogen clearance method with acutely implanted platinum electrodes. Hypoxia (PaO_2 35.3±2.4 Torr) and hypercapnia ($PaCO_2$ 68.1±5.1 Torr) increased basal CBF from 76.3±9.0 ml/100g/min to 168.1±20.1 ml/100g/min and 162.4±31.9 ml/100g/min respectively. The sulphonylurea tolbutamide (1mM in 1%DMSO) had no significant effect on CBF in hyperoxia or in hypercapnia. However, it attenuated the increase of CBF during hypoxia by 66 ± 11 % ($p<0.01$). This suggests that opening of tolbutamide-sensitive potassium channels may be involved in the process of hypoxic vasodilation in the rat cerebral cortex.

Keywords: Cerebral blood flow, sulphonylurea, ATP-sensitive K^+channel, hypoxia, hypercapnia.

Introduction

Hypoxia and hypercapnia both markedly increase cerebral blood flow (CBF) by mechanisms which remain unresolved. In the case of hypoxia, it has been suggested that potentiation of CBF may be at least partially mediated by a rise in the extracellular K^+ concentration ($[K^+]_o$), since increasing $[K^+]_o$ to between 7 and 15mM dilates cerebral arteries *in vitro* [6], and $[K^+]_o$ has been reported to rise in the rat cerebral cortex during ischaemia [4] *in vivo*. Activation of ATP-sensitive K^+-channels (K_{ATP} channels), or inhibition of the Na/K ATP-ase, in response to reduced metabolism may be responsible for this elevation of $[K^+]_o$. In pancreatic ß-cells and cardiac muscle, sulphonylureas such as tolbutamide and glibenclamide act as potent and selective inhibitors of K_{ATP} channels [1]. To explore the possible role of K_{ATP} channels in the regulation of CBF, we have therefore examined the effects of these drugs on CBF during hypoxia and hypercapnia.

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Methods

Adult albino rats (300-400g, n=14) were anaesthetized with 1-2% halothane in 100% oxygen, tracheostomized and artificially ventilated. The left femoral artery and vein were then cannulated for recording arterial blood pressure (ABP, Sensor 88400, Norway) and infusing fluid (1-2ml/hr i.v. of 1.5% HCO_3 in Ringer). Rats were placed in a stereotaxic frame, and a small (2mm) craniotomy was made in the parietal bone lateral to the midline. The dura was removed and a dual stainless steel catheter (each barrel 425µm in diameter) was inserted 1-1.3mm into the cortex under stereotaxic control. One barrel of the catheter contained a 125µm thick platinum electrode coated in teflon. The second barrel, immediately adjacent to the first, was used for infusion of drugs. After surgery, halothane was replaced by 1-2% enflurane in oxygen since enflurane is known to have a smaller effect on CBF. Body temperature was maintained at 38±1° C. Data were not recorded until 30 min after insertion of the platinum electrode. In several experiments the position of the H_2 electrode was confirmed by histological examination of fixed brain sections to be within the gray matter.

Cerebral blood flow was measured using the hydrogen clearance method [2]. Briefly, this method consists of measuring the rate of washout of inspired H_2 using an implanted platinum electrode to measure H_2 concentration. The platinum electrode, contained within one barrel of the catheter, was held at a potential of +250mV and the current resulting from H_2 oxidation was measured with a high input impedance (100 MΩ) amplifier. For each measurement of CBF hydrogen (*ca.* 10-20%) was administered in the inspirate for 15-60 s. Data were acquired on-line and analysed using LABVIEW software run on a Quadra 950 Apple Macintosh computer. CBF was obtained from the decay phase of the hydrogen clearance curve where CBF(ml/100g/min) = $0.693/\lambda \times T_{1/2} \times 60s \times 100g$, where λ is the partition coefficient for H_2 and is taken to be 1 and $T_{1/2}$ is the time in seconds for the current to decay by half, and 0.693 is a constant [see ref. 2].

Condition	CBF (ml/100g/min)	PaO_2 (Torr)	$PaCO_2$ (Torr)	ABP (mmHg)	pH	n
Hyperoxia/Control	76.3±9.0	216.2±18.3	35.8±0.5	122.0±7.4	7.48±0.02	8
Hyperoxia+Tb	75.6±7.2	193.4±28.2	35.1±0.9	99.0±6.4	7.44±0.01	5
Hypoxia	168.1±20.1	35.3±2.4	35.6±0.5	90.0±3.0	7.45±0.02	8
Hypoxia+Tb	105.5±14.8	36.1±1.4	36.0±0.5	80.2±7.4	7.45±0.02	8
Hypercapnia	162.4±31.9	146.9±16.7	68.1±5.1	90.0±6.2	7.23±0.02	5
Hypercapnia+Tb	163.1±18.1	209.3±17.6	66.1±3.7	95.0±7.4	7.21±0.04	7

Table 1. Mean data of $PaCO_2$, PaO_2, arterial pH, ABP and corresponding CBF values under various conditions ± tolbutamide (Tb). Errors are ± S.E.M.

Arterial blood was sampled after each hydrogen clearance curve and analysed for pH and blood gases using a blood gas analyser (ABL505).

Ventilation was adjusted to ensure a normal arterial PCO_2 ($PaCO_2$, 33-39 Torr), except during hypercapnia: during hypoxia it was often necessary to supplement the inspired gas mixture with CO_2 to hold the $PaCO_2$ constant. Hypoxia was induced by partially replacing oxygen in the inspirate with nitrogen, and CO_2 was added to induce hypercapnia. Hypoxia and hypercapnia were applied for *ca.* 30 and *ca.*10 minutes respectively. To minimise the possibility of uncontrolled cerebral hypoxia during prolonged experiments, mild hyperoxia (PaO_2 ~200 Torr) was used as the control condition rather than allowing the rat to breathe atmospheric air alone. Experiments in which no hypoxic or hypercapnic increase in CBF was observed, or in which the blood pressure fell outside the limits of autoregulation (i.e. 70 - 140 mmHg) were discarded.

Drugs, or vehicle, were infused at a rate of 0.5µl/min for 1-2 hours. A 30 min period for equilibration was allowed before measurement of CBF. Tolbutamide (1mM) and glibenclamide (10µM) were dissolved in 1% dimethyl sulfoxide (DMSO) in 0.9% saline.

Data are expressed as mean±S.E.M. and statistical comparisons were performed using a paired two tailed t-test. Significance was taken to be $P<0.05$.

Results

Table 1 gives mean results of experiments in which CBF was measured during hyperoxia, hypoxia or hypercapnia in the presence and absence of tolbutamide. The same data are shown diagrammatically in Fig. 1, where CBF is expressed as a percentage of its value under control conditions. Basal CBF varied between 50-117 ml/100g/min, with a mean flow of 76.3 ml/100g/min. This increased over two-fold to 168.1 ml/100g/min ($p<0.01$) when PaO_2 was reduced to 35 Torr. The sulphonylurea tolbutamide (1mM) had no significant effect on basal CBF but reduced hypoxic CBF by 66 ± 11% ($p<0.01$). The reversibility of the effect of tolbutamide was tested by stopping infusion of the drug. In 3 out of 4 rats tested the hypoxic response reversed completely within 20-30 mins; partial recovery was observed in the fourth rat.

Raising $PaCO_2$ from its control level of 35 Torr to 68 Torr also approximately doubled CBF, to 162.4 ml/100g/min. However, in contrast to the sulphonylurea-mediated reduction of flow in hypoxia, tolbutamide was without effect on CBF during hypercapnia. Similar results were obtained with glibenclamide (10µM) which also attenuated CBF during hypoxia (73 ± 13 % attenuation, n=3) but had no effect in hypercapnia (n=3).

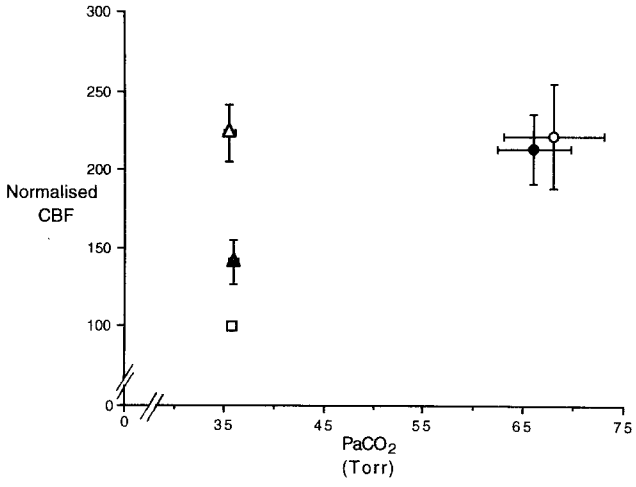

Figure 1. Summary diagram of CBF with control/hyperoxic CBF normalised to 100. Open square is control/hyperoxia, open triangle is hypoxia, open circle is hypercapnia, filled symbols are in the presence of 1mM tolbutamide.

Although ABP fell during hypoxia and hypercapnia (Table 1), it was still maintained within the limits of autoregulation (70-140 mmHg), so changes in ABP are unlikely to contribute to changes in CBF. The plasma pH remained stable throughout hypoxia but, as expected, fell in response to hypercapnia.

The vehicle in which the sulphonylurea was delivered (1% DMSO in 0.9% saline) did not affect CBF under control conditions, or reduce the increases in CBF produced by hypoxia or hypercapnia.

Discussion

The results described here demonstrate that the sulphonylureas tolbutamide and glibenclamide

attenuate the increase in CBF produced by hypoxia, but not that induced by hypercapnia. Dilation of the coronary vasculature during hypoxia is also blocked by glibenclamide [3]. The possibility that the reduction in hypoxic vasodilation induced by sulphonylureas results from an effect of the vehicle can be excluded, because the vehicle was without effect when infused alone, under either control conditions or during hypoxia or hypercapnia. For the same reason, a volume effect may be ruled out.

Tolbutamide only decreased the hypoxic increase in CBF by about 66%. There are two possible explanations for the failure of the drug to reduce CBF to basal levels. First, tolbutamide-insensitive mechanisms may account for around one third of the hypoxic vasodilation. Secondly, the concentration of tolbutamide at its target site may not be sufficient to produce a maximal response. It is well known that plasma proteins bind sulphonylureas with high affinity and reduce the free plasma concentration to less than 10% of total [8]. Thus if tolbutamide reaches some of its target sites via the microcirculation, its effective concentration may be substantially lower than that infused.

Tolbutamide was without effect under control conditions indicating that it is selective for processes activated during hypoxia. This would be consistent with an action of the drug on K_{ATP} channels. K_{ATP} channels located in vascular smooth muscle, endothelium, neurons or even glial cells may be the primary target for tolbutamide *in vivo*. The presence of K_{ATP} channels in rat cerebral smooth muscle is controversial. Although one study has shown that rat cerebral arteries *in vitro* are not dilated by the K-channel openers cromakalim and pinacidil [7], in a recent study the K-channel opener RP52891 dilated rat cerebral arterioles *in situ*, an effect which was blocked by 1μM glibenclamide [5]. The lack of effect of tolbutamide on control (hyperoxic) CBF in this study is consistent with the finding that glibenclamide had no effect on the resting tone of pial arteries *in situ* [5]. It is worth pointing out, however, that a specific action of either K-channel openers or sulphonylureas on K_{ATP} channels in cerebral smooth muscle has not yet been established. In addition to an action of sulphonylureas on smooth muscle, hypoxic vasodilation in the rat brain may be due to a sulphonylurea-sensitive K^+ efflux from neurones or glial cells, since $[K^+]_o$ rises during ischemia in the rat cortex [4] and $[K^+]$ (7-15mM) dilates rat cerebral arteries [6].

In contrast to the hypoxic-mediated increase in CBF, the increase induced by hypercapnia was insensitive to sulphonylureas. This indicates that a different mechanism mediates hypercapnic vasodilation and further suggests that this process does not involve activation of K_{ATP} channels.

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