IJMS
Vol. 27, No. 1, March 2002

Original Article

Role of Nitric Oxide and ATP-Sensitive K^+ Channels in Regulation of Basal Blood Flow and Hypercapnic Vasodilatation of Cerebral Blood Vessels in Rabbit

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Abstract

Background: The mechanisms underlying cerebral hypercapnic vasodilatation are not fully understood.

Objective: To investigate the role of nitric oxide (NO) and ATP-sensitive potassium (K_{ATP}) channels in basal blood flow regulation and hypercapnia-induced vasodilatation in rabbit cerebral blood vessels.

Methods: The change in cerebral blood flow was measured by a laser Doppler flowmeter in 18 New Zealand white rabbits, in two groups, under general anesthesia with sodium pentobarbital. N-omega-nitro-L-arginine methyl ester (L-NAME) and glibenclamide were administered locally and systemically before and during induction of hypercapnia.

Results: The change in cerebral blood flow was not significant following local and systemic L-NAME administration, showing a non-significant role of local and systemic NO in regulation of rabbit basal cerebral blood flow. Hypercapnia increased cerebral blood flow by 17.3±4.4% before and 17.3±5.8% after local, and 5.8±3.2% ($p<0.05$) after systemic L-NAME administration. The change in cerebral blood flow was not significant after local and systemic administration of glibenclamide indicating a lack of K_{ATP} channel role in basal blood flow regulation. Hypercapnia increased cerebral blood flow by 27.2±8.7% before and 24.7±6.4% after local, and 49.3±9.7% after systemic administration of glibenclamide (p: NS in both cases).

Conclusion: Regional NO production had no role in basal cortical blood flow regulation and systemic NO contributed to 66% increment in cerebral blood flow during hypercapnia. Also, the K_{ATP} channels did not mediate the effect of NO or other vasodilators responsible for increasing cerebral blood flow during hypercapnia.
Iran J Med Sci 2002; 27(1): 22-29

Keywords • Cerebral blood flow • Hypercapnia • ATP-sensitive potassium channels • Nitric oxide

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Introduction

It is well documented that the brain is able to maintain its blood flow constant over a wide range of arterial 0blood pressures (BP). It is believed that this autoregulation is mainly through metabolic factors such as carbon dioxide, hydrogen ions and oxygen, with CO_2 as the most potent one.[1,2] However, the mechanisms through which CO_2 functions is not fully understood. Recent investigations have revealed the role of nitric oxide (NO) in increasing cerebral blood flow by different mechanisms.[3-5] In mice, inhibition of NO production by local application of N(G)-nitro-L-arginine (L-NNA) reduced cortical basal blood flow by 25%, and the hypercapnia-induced blood flow increments by 60%.[6] In rat, L-NNA has been shown to inhibit hypercapnic vasodilatation by 90%.[7] In another study on rat, 77% of the rise in blood flow caused by hypercapnia was inhibited by systemic L-NNA treatment.[8] Other studies on the rat using different NO production inhibitors systemically, have shown an attenuation of vasodilator response to hypercapnia by 42%, 48%, 55% and 70%.[9-12] In the cat, a substantial reduction in CO_2 response has been observed by intravenous administration of nitric oxide synthase (NOS) inhibitor—N-omega-nitro-L-arginine methyl ester (L-NAME).[13] There is evidence showing that the contribution of NO to BP (and therefore, vascular resistance) regulation differs between rat and rabbit; as the BP was increased by 20% in response to 3 mg/kg L-NAME administration in rabbit[14] compared to 67% in rat (Ferrell, Lockhart and Najafipour, unpublished observations). A study on rabbit cortical blood vessels, indicated a 25% increase in arteriolar diameter by moderate hypercapnia, however, cortical blood flow (CBF) was not recorded in this study directly and also L-NNA was applied only topically, hence, without investigating the origin of NO.[15] On the other hand, activation of K_{ATP} channels causes hyperpolarization and relaxation of vascular smooth muscle cells, leading to vasodilatation, which mimics the effect of NO.[16-18] Some investigations have demonstrated that K_{ATP} channels are involved in NO-induced vasodilatation in cat but others could not show such involvement in piglets.[19-20] We did not find any research work, addressing the role of K_{ATP} channels in rabbit CBF regulation during normocapnia and hypercapnia. An increase in cerebral arteriolar diameter was left unaffected by glibenclamide in higher levels of hypercapnia, while it was reduced by 36% in lower levels.[15] Due to interspecies differences and paucity of information on rabbit cortical blood vessels response to hypercapnia, we conducted this study to investigate the role of NO and K_{ATP} channels in basal blood flow regulation and in hypercapnic vasodilatation, and also to address the issue whether NO functions through K_{ATP} channels in this animal.

Materials and Methods

Animal preparation

Experiments were conformed to the University Research Council guidelines for conducting animal studies. Eighteen New Zealand white rabbits weighing 2-3 kg were anesthetized initially by injection of Hypnorm™ (0.15 ml/kg, i.m., Janssen, UK) and diazepam (1.5 mg/kg, i.p.). During the surgical procedure, anesthesia was continued with a respiratory gas mixture of 1% halothane, 30% O_2, and 70% N_2O. On completion of surgery, the gaseous anesthesia was discontinued and animals were kept anesthetized by sodium pentobarbital (30 mg/kg initially followed by 10 mg/kg/hr afterwards) via a cannula, inserted into the femoral vein. Trachea was cannulated, with animals breathing spontaneously throughout the surgical procedure, and artificially ventilated after using muscle relaxant (see below). Deep anesthesia was maintained throughout the surgery as judged by the absence of withdrawal response to the pinch stimulus applied to the hind limb. BP was monitored throughout the experiment via a cannula inserted into the femoral artery and blood samples were collected when needed.

The scalp was shaved in the parietal area and a 1-1.5 cm incision was made longitudinally to the right of the midline. Cranial bone was removed over an area of about 1 cm^2 and the dura was lifted and cut around carefully to get access to cortical blood vessels. The skin tips around the area were lifted and secured to metal rods to make a CSF pool over the exposed area of the cortex. The pool was filled and continuously superfused with artificial CSF at 37 ^oC aerated with 95% O_2 and 5% CO_2 at a flow rate of 1-1.5 ml/min. CSF composition was, 120 mM NaCl, 2.8 mM KCl, 22 mM $NaHCO_3$, 1.45 mM $CaCl_2$, 1.0 mM Na_2HPO_4, and 0.876 mM $MgCl_2$. The pH and pCO_2 of the superfusing medium were 7.43±0.065 and 32.1±3.6 mm Hg respectively, in the L-NAME group, and 7.41±0.035 and 32.5±0.6 mm Hg respectively, in the glibenclamide group. Control values for arterial pH and pCO_2 were 7.35±0.017 and 33.4±1.5 mm Hg (n=5) respectively, when animals were on spontaneous breathing. A 0.9 mm diameter fibre optic probe connected to a laser doppler flowmeter (Moor Instruments; model MBF3D, Axminster, UK) provided a measure of relative changes in the blood flow of tissues beneath the tip of the probe. The probe was positioned just above the pial surface. At the end of the surgical procedure, relaxation was induced by i.v. injection of 0.4 mg/kg pancronium bromide.[21] The animals were connected to a respirator functioning at a rate of 40/min, and a tidal vol-

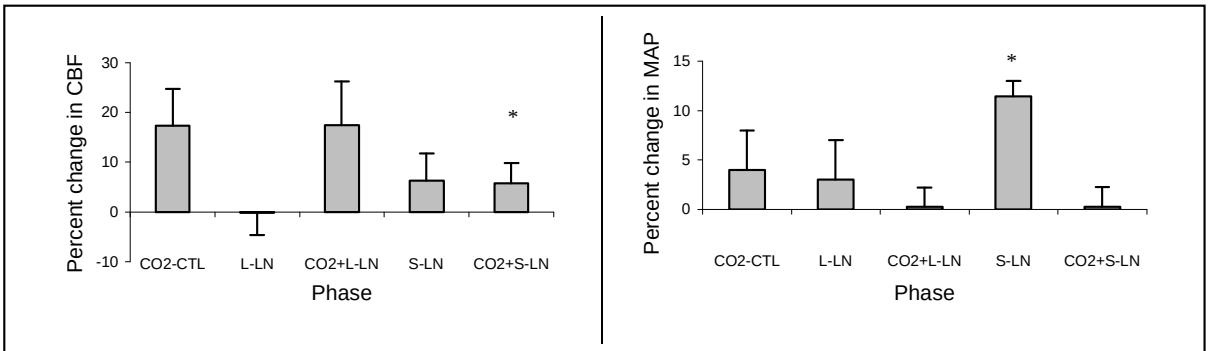

Figure 1 (Left): Alterations in cortical blood flow; and **(Right):** Alterations in mean arterial pressure, due to hypercapnia in control conditions (CO_2-CTL), local L-NAME administration (L-LN), Hypercapnia after local L-NAME (CO_2+L-LN), systemic L-NAME administration (S-LN), and hypercapnia after systemic L-NAME (CO_2+S-LN) in 8 rabbits. Hypercapnia induced vasodilatation significantly reduced after systemic L-NAME. *=p<0.05 compared to control. Blood pressure significantly increased after systemic L-NAME. * =p<0.05 compared to local L-LNAME.

ume of 20-25 ml, based on animal weight.[22] Lack of change in BP in response to pinch stimulus on hind limb confirmed the deep level of anesthesia in paralyzed animals. In order to minimize the effects of surgical stress on blood flow or BP we allowed a period of at least one hour to lapse prior to performing any test procedure. Hypercapnia was induced by connecting the respirator circuit inlet to a 100-litre Douglas bag containing 6.5% CO_2 in air for 10 minutes. We checked the accuracy of CO_2 concentration in the bag by a medical gas analyser (LB2, Beckman, USA).

The following agents were used: L-NAME and glibenclamide (Sigma, UK), and pancronium bromide (Darou Pakhsh, Iran). For systemic administration, L-NAME (30 mg/kg) was dissolved in 2 ml of normal saline and injected through the femoral venous cannula. For topical application L-NAME was dissolved in artificial CSF to a concentration of 1 mM. For systemic administration, glibenclamide (3 mg/kg) was dissolved in 1 ml of 96^o ethanol and added drop by drop to 15-20 ml of normal saline to make a homogenous solution. This solution was continuously infused via the femoral venous cannula to the related group of animals by a rate of 1.5 ml/min. For topical application, glibenclamide was dissolved in ethanol and then added to artificial CSF to a concentration of 1 µM.[15]

Experimental protocol

Two groups of animals were studied; In the first group (L-NAME group, n=8), at the end of resting period, while the animal was on normal air (normocapnia), arterial BP and CBF were recorded and a 1 ml of arterial blood sample was collected. Then experiment was continued in five steps as follows:

1. The animal was shifted to hypercapnia, and at the end of 10 minutes period BP and CBF were recorded.
2. The animal was shifted to normocapnia and after 15 minutes (when BP and CBF returned back to the basal values), artificial CSF perfusate was replaced with CSF containing 1 mM L-NAME. At the end of at least 30 minutes of topical L-NAME application, BP and CBF were recorded.
3. The animal was shifted to hypercapnia for another period of 10 minutes, and topical L-NAME application was continued. At the end, BP and CBF variables were recorded.
4. The animal was shifted to normocapnia, and following a 15 minutes stabilizing period, BP and CBF were recorded. L-NAME (30 mg/kg) was injected systemically while topical L-NAME was continued and after 30 minutes, BP and CBF were recorded.
5. The animal was shifted to hypercapnia and after 10 minutes, BP and CBF were recorded, while topical L-NAME application was continued during this phase as well.

An arterial blood sample was collected at the end of each of the above-mentioned phases from five animals.

In the second group of animals (glibenclamide group, n=10), at the end of resting period, while on normal air (normocapnia), BP and CBF were recorded and arterial blood sample was collected. Then, experiment was continued in five steps as mentioned for L-NAME group except that topical (1 µM) and systemic (3 mg/kg) glibenclamide were used instead of L-NAME in steps 2 and 4.

Blood samples along with samples of artificial CSF superfusate were kept in ice and the pCO_2, pO_2, and pH were measured at the end of experiment

Table 1: Arterial pO_2, pCO_2 and pH values in normocapnic and hypercapnic conditions before, and after local and systemic L-NAME administration in 5 rabbits.

	State					
	Normocapnia			Hypercapnia		
Factor	**CTL**	**L-LN**	**S-LN**	**CTL**	**L-LN**	**S-LN**
pCO_2 (mm Hg)	30.2±3.1	30.9±3.7	32.2±3.7	48.5±1.6	48.8±1.7	52.9±1.1
pH	7.45±0.04	7.41±0.03	7.38±0.04	7.24±0.02	7.19±0.02	7.16±0.02
pO_2 (mm Hg)	76.1±2.6	72.2 ±2.7	85.7±11	88.6±2.7	99.9±8.2	82.5±8.3

CTL: control conditions (before L-NAME); L-LN: local L-NAME; S-LN: systemic L-NAME. Hypercapnia significantly increased arterial pCO_2 and reduced arterial pH, but none of the values of three variables are significantly different between 3 stages of normocapnia or between 3 stages of hypercapnia.

using a clinical blood gas analyser (AVL 995, Copenhagen, Denmark).

Termination of the experiment was achieved by intravenous administration of 1 M KCl. Alterations in blood flow, BP, and vascular resistance are expressed as percentage change from control values occurring immediately before each test procedure. The biological zero values were measured as described previously and subtracted from the flow values before calculation of percentage changes in blood flow.[23] Mean arterial pressure was calculated by adding one-third of pulse pressure to the diastolic pressure.[1] Vascular resistance was estimated by dividing mean arterial pressure by blood flow (*i.e.,* R=P/Q).

In the Figures and Tables, the values are expressed as Mean±SEM. Means were compared using one-way ANOVA followed by Tukey's HSD as *post hoc* test. P-values less than 0.05 were considered significant.

Results

Effect of L-NAME administration

In the first group of animals (n=8), the effect of topical and systemic administration of L-NAME was investigated on CBF during control (basal) conditions and hypercapnia.

Figure 1 (Left) shows changes in CBF due to hypercapnia before and after topical and systemic L-NAME administration. Hypercapnia increased CBF by 17.3±5.7% of control conditions. This increment was 17.3±7.4% after local (p: NS) and 5.8±3.8% after systemic L-NAME administration ($p<0.05$). Resting CBF showed a very small reduction during local L-NAME superfusion, however, it increased during 30 minutes of systemic L-NAME administration by 6.2±5.2%. Blood flow values were 179.4±15.8 arbitrary units in control conditions, 176.5±15.5 units after topical and 192.5±15.7 units after systemic L-NAME administration.

Figure 1 (right) indicates changes in BP due to systemic L-NAME administration. BP showed small and non-significant changes during three steps of hypercapnia (control, after local and after systemic L-NAME). BP was only increased by 3±3.2% during local L-NAME administration. However, it was elevated by 11.4±2.3% by systemic L-NAME administration ($p<0.03$ compared to local effect). Absolute BP values were 81±5.9 mm Hg in control conditions, 83.3±5.2 mm Hg after topical and 92.9±5.3 mm Hg after systemic L-NAME administration. Cerebral vascular resistance changed by 9.4±7% during local (from 0.45±0.05 to 0.49±0.06 mm Hg/flow unit; p: NS), and by 3.7±4.1% during systemic L-NAME administration (from 0.47±0.05 to 0.49±0.06 mm

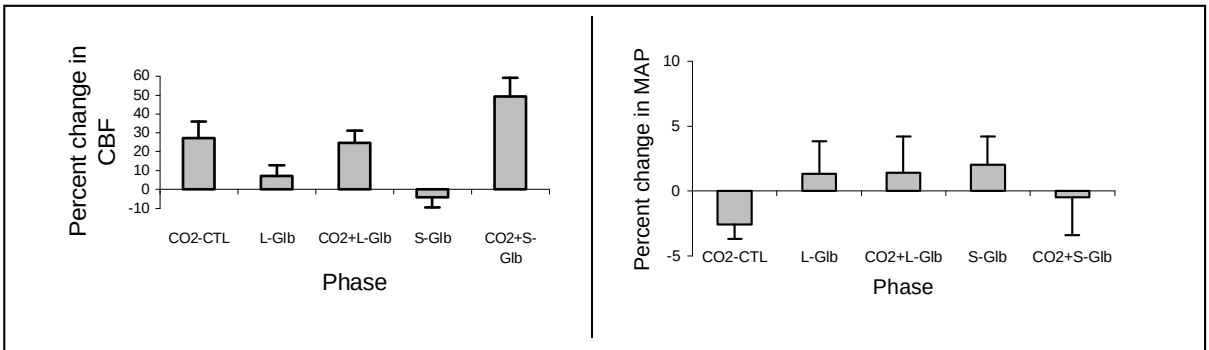

Figure 2 (Left): Alterations in cortical blood flow; and **(Right):** Alterations in mean arterial pressure, due to hypercapnia in control conditions (CO_2-CTL), local glibenclamide administration (L-Glb), Hypercapnia after local glibenclamide (CO_2+L-Glb), systemic glibenclamide administration (S-Glb), and hypercapnia after systemic glibenclamide (CO_2+S-Glb) in 10 rabbits. There was no significant difference between blood flow or blood pressure changes in three stages of hypercapnia or between local and systemic glibenclamide effects

Table 2: Arterial pO_2, pCO_2 and pH values in normocapnic and hypercapnic conditions before, and after local and systemic glibenclamide administration in 5 rabbits.

	State					
	Normocapnia			Hypercapnia		
Factor	CTL	L-GL	S-GL	CTL	L-GL	S-GL
pCO_2 (mm Hg)	34.8±3.3	36±3.2	33.2±3.5	50.0±2.6	49.2±1.9	49.4±1.5
pH	7.38±0.03	7.36±0.05	7.39±0.04	7.15±0.02	7.13 ± 0.03	7.13±0.03
pO_2 (mm Hg)	79.3±8.6	73.4±7.8	75.2±8.1	102±6.1	99.3 ± 12	105±8.5

CTL: control conditions (before glibenclamide); L-GL: local glibenclamide; S-GL: systemic glibenclamide. Hypercapnia significantly increased arterial pCO_2 and reduced arterial pH, but none of the values of three variables are significantly different between 3 stages of normocapnia or between 3 stages of hypercapnia.

Hg/flow unit; p: NS).

As hypercapnia causes a reduction of arterial pH, that may affect CBF in addition to that caused by hypercapnia *per se*, arterial blood samples were taken from five animals during the course of experiment to assess if there were any difference in pH or blood gas parameters among different stages of normocapnia or hypercapnia. Table 1 summarizes the results.

Effect of glibenclamide administration

In the second group of animals (n=10), the effect of topical and systemic administration of glibenclamide was investigated on CBF during control (basal) conditions and during hypercapnia.

Figure 2 (left), shows changes in CBF due to hypercapnia before and after topical and systemic administration of glibenclamide. Hypercapnia increased CBF by 27.2±8.2% under control conditions. This increment was 24.7±6.4% after local and 49.3±9.7% after systemic administration of the drug (p: NS in both cases). Resting CBF showed non-significant changes during local or systemic glibenclamide administration. Blood flow values were 195±14.7 arbitrary units in control conditions, 210±18 units after topical and 199±13 units after systemic administration of glibenclamide.

Figure 2 (right), indicates changes in BP due to hypercapnia before and after topical and systemic glibenclamide administration. BP showed small and non-significant changes during three steps of hypercapnia (control, after local and after systemic administration of glibenclamide). Administration of local and systemic glibenclamide also had non-significant effects on resting BP. Absolute mean arterial pressure values were 77.3±3.7 mm Hg in control conditions, 78.3±5.8 mm Hg after topical and 80.2±5.2 mm Hg after systemic administration of glibenclamide.

For the same reason, as described previously, arterial blood samples were taken during the course of experiment from five animals of the second group. Table 2 summarizes the results of samples' analyses.

Discussion

Role of NO in basal cortical blood flow regulation

In the present study, an NO production inhibitor, L-NAME, was used to assess the role of NO in regulation of basal CBF in the rabbit. Only a small change in basal CBF occurred during 30 minutes of L-NAME superfusion (Fig.1 (left)). Therefore, local release of NO, probably has no role in basal CBF regulation in this vascular bed. The small increment in systemic BP during this period (Fig.1 (right)) shows that L-NAME had been absorbed a little through the small area of cortex exposed to it and that there had been no interference by BP change on blood flow. In contrast, when L-NAME was administered systemically, more than 11% increase in BP did occur. CBF was also increased. This parallel increase again shows the non-significant role of NO in resting CBF regulation in rabbit (as non-significant change in vascular resistance due to L-NAME administration also confirms this (see results)). Meantime, an important role for NO in regulation of vascular resistance to most other vascular beds is evident because of increase in systemic BP (Fig.1 (right)). In contrast, in a similar study in mice and rats, 25% and 38% reduction in resting CBF has been shown after local L-NNA administration, respectively.[6,7] The results of this study are in agreement with those reported by Fabricius and Lauritzen in rats, in which L-NNA application failed to reduce basal CBF, and also is in agreement with the study of Faraci in rabbit in which cortical arteriolar diameter was not changed significantly by local application of L-NNA.[8,15]

Role of NO in hypercapnic vasodilatation

More than 17% increase in blood flow during hypercapnia (Fig.1 (left)) in the presence of a very small change in BP (Fig.1 (right)) indicates that in rabbit, like many other species such as mice, rats, and pigs, cortical blood vessels are dilated in response to increased arterial pCO_2.[3,6,8] This increment was 17.5% (p: NS) after local L-NAME administration and 5.5% ($p<0.05$) after systemic L-NAME administration (Fig.1 (left)). The non-significant change in response to local L-NAME but about 66% reduction in response to systemic L-NAME administration implies that NO responsible for this increment in blood flow, is not produced locally but probably has a systemic vascular origin. NO is produced

in many vascular beds, however, for the very short half life (less than five seconds) it has,[24] the amount of NO that reaches cortical blood vessels is below the threshold required for vessel dilatation, and thus, it probably does not affect the cortical blood vessels in resting conditions. Nevertheless, in hypercapnic conditions in which a dramatic amount of NO is released, the amount reaching the cortical blood vessels is enough to produce a significant vasodilatation. Therefore, a reduction of 66% in vasodilatory response to hypercapnia after systemic L-NAME, and the lack of local L-NAME effect is in favor of systemic origin of NO for the hypercapnic vasodilatation in cortical blood vessels of rabbits. pCO_2 and pH of artificial CSF have been in the same range of their arterial value (see methods section); hence, the cortical blood vessels had been exposed to a physiological condition regarding these two important variables, which affect vascular responsiveness. The arterial pCO_2 values may seem smaller than pCO_2 values in humans. Other investigators have also shown smaller arterial pCO_2 values in control conditions in rat (pCO_2 range: 34.1-36.3 mm Hg) and rabbit (pCO_2 = 32±1 mm Hg).[15,25] It may be, then, argued that L-NAME, when used topically, could not pass the blood brain barrier and reach endothelium to block NO synthesis. However, using the same method of local application of NOS inhibitors, other investigators have shown a significant reduction in vasodilatory response to hypercapnia in mice, rats and rabbits.[6,8,15] On the other hand, the acetylcholine-induced rise in CBF, which is known to exert its effect exclusively by stimulating endothelial NO, was completely blocked by topical application of L-NNA.[8,15] In a study on mice, hypercapnia caused a 50% increase in CBF, from which 60% was reduced by local L-NAME administration.[6] These results are different from the smaller vasodilatation (17.3%) observed in this study and from the non-significant local L-NAME effect. Also in rat, a 70% increase in CBF has been observed due to hypercapnia; four-folds compared to what was found in the current study.[8] From this figure, 52% was reduced by local L-NNA and 77% was reduced by systemic L-NNA administration. Therefore, in contrary to the results of this study, in mice and rats more than 50% of response was due to local NO production, and NO, probably with both local and systemic origins, was responsible for hypercapnic vasodilatation in those species.

Role of K_{ATP} channels in basal cortical blood flow regulation

The superfusion of glibenclamide, a specific K_{ATP} channel blocker, [26] over the cortex for 30 minutes had a non-significant effect on basal CBF (Fig. 2 (left)). Also, systemic infusion of glibenclamide caused a very small reduction in basal CBF during 30 minutes (Fig. 2 (left)). Therefore, K_{ATP} channels have no role in blood flow regulation at rest and apparently none of the factors regulating basal CBF in the rabbit works through K_{ATP} channels. On the other hand, systemic BP did not change significantly due to local or systemic administration of glibenclamide (Fig. 2 (right)). This may show that vascular resistance, neither in cortical blood vessels nor in other vascular beds, is affected by K_{ATP} channel blockage significantly. The results of this study are in agreement with other studies performed on pigs, piglets, and rats in which these channels were shown to have no role in regulation of cerebral vessel tone.[5,20,27,28]

Role of K_{ATP} channels in hypercapnic vasodilatation

Increments in CBF due to hypercapnia after local or systemic glibenclamide were not significantly different from control hypercapnia (Fig.2 (left)). There was no significant change in BP during three stages of hypercapnia as well (Fig.2 (right)). Therefore, these channels may have no role in hypercapnic vasodilatation of the cerebral vascular bed. It is also concluded that NO which was shown to be responsible for 66% increase in CBF during hypercapnia (Fig.1 (left)) does not act through K_{ATP} channels. These results are somehow different from those reported in another study on rabbits that showed that glibenclamide was able to reduce the hypercapnia-induced increase in arteriolar diameter from 25% to 16%.[15] The difference may be due to different levels of hypercapnia ($paCO_2$ =54 mm Hg in that study *vs* 49 mm Hg in the current study) or different techniques of vasodilatation assessment in the two studies (flow measurement in present study and arteriolar diameter measurement in that study). The reports by Faraci and Armstead in rabbit and newborn pigs also show that glibenclamide is able to reach endothelium when used topically because the vasodilatory responses of cerebral arterioles to hypercapnia or hypoxia were significantly reduced by 1µM topical glibenclamide application in those studies. [15,29] Although not statistically significant, CBF almost doubled due to hypercapnia after systemic glibenclamide administration (Fig.2 (left)). The reason for this, is not still known, as it was expected that K_{ATP} channel blocking would reduce the response.

Conclusion

Overall, the results of this study show that in rabbit cortical blood vessels, neither NO, nor K_{ATP} channels have a role to play in regulation of basal vascular tone. This vascular bed dilates in response to hypercapnia, however, in contrast to mice and rats, no locally produced NO contributed to this vasodilatation, though systemically produced NO played a sig-

nificant role. K_{ATP} channels also did not mediate hypercapnic vasodilatation of these vessels. Vasodilatation due to NO was not also mediated through K_{ATP} channels in this animal.

Acknowledgement

This project was supported by the Kerman University of Medical Sciences and Health Services, Kerman, Iran.

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In the Name of God

CALL FOR ABSTRACTS

8th Razi (Rhazes) Medical Sciences Research Awards

December, 2002

Relying upon the assistance of the Almighty God, the 8th Razi Medical Sciences Research awards is to be held on December, 2002 in the following five sections:

1 Papers published in peer reviewed Iranian and foreign scientific journals
2 Student theses from Iranian universities published in one of scientific Journals.
3 Innovations and inventions
4 Medical research centers.
5 Scientific-research Journals approved by the Publications committee of the Ministry of Health and Medical Education.

Eligibility for participation

To submit a copy of the published paper along with the project report
To submit a copy of the students thesis along with the related papers.
To submit issues of the scientific journals published from March 20, 2001 to April 2002.
To submit the related form for the Research Centers Section.
A full description around inventions/innovations along with the invention registration certificate

Notes

1st The submitted papers will be assessed among specialized medical fields of basic, clinical, pharmaceutical, nutrition, health, dentistry, rehabilitation and medical equipment sciences.
2nd The papers should have been published recently (March 2000 or afterwards).
3rd The students section includes whom introduced by deputy of research affairs of their universities, (Medical sciences universities can present up to 3 students).
4th The theses should must be fulfilled more recently than March 2000
5th Applicants holding BS degree or above from Iran
6th F Research Centers are introduced by deputy research affairs of medical science universities. So, the related form should be filled out and submitted along with other documents.
7th G The documents shall not be returned to the applicants
8th H. Acceptance proof of papers will not be acceptable.

Further information is available for interested applicants at the secretariat of Razi festival, National Research Center of Medical Science, deputy of research affairs of medical sciences Universities or at http://nrcms.org

Deadline for submission of documents:

September 27, 2002

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