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Role of nitric oxide in the effects of hypoglycemia on the cerebral circulation in awake goats

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Abstract

This study was performed to examine the role of nitric oxide in the effects of hypoglycemia on the cerebral circulation. Hypoglycemia was induced with insulin and its effects on cerebral blood flow (measured with an electromagnetic flow transducer placed on the internal maxillary artery) were studied in awake goats under control conditions and after administration of the nitric oxide synthesis inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 47 mg/kg). Also, cerebrovascular reactivity to vasodilator stimuli was examined during insulin-induced severe hypoglycemia, before and after L-NAME treatment. In five animals under control conditions (glycemia = 90 ± 7 mg/dl, cerebral blood flow = 64 ± 4 ml/min, mean systemic arterial pressure = 102 ± 4 mmHg, cerebrovascular resistance = 1.62 ± 0.11 mmHg/ml per min and heart rate = 73 ± 6 beats/min), insulin decreased glycemia: when hypoglycemia was moderate (glycemia = 46 ± 2 mg/dl) or severe (glycemia = 26 ± 1 mg/dl) cerebral blood flow increased by 25 ± 4% and 47 ± 6%, and cerebrovascular resistance decreased by 18 ± 3% and 34 ± 4%, respectively. Under basal conditions, L-NAME did not affect glycemia but reduced resting cerebral blood flow by 37 ± 2%, increased mean arterial pressure by 33 ± 2% and decreased heart rate by 28 ± 3%; after L-NAME, both moderate and severe hypoglycemia did not alter significantly resting cerebral blood flow and cerebrovascular resistance. In five other goats, L-NAME, administered during severe hypoglycemia, abolished the increase in cerebral blood flow, and increased cerebrovascular resistance and mean arterial pressure over the control (normoglycemic) values. In these animals with severe hypoglycemia, acetylcholine (0.01–1 μg), isoproterenol (0.03–3 μg) and diazoxide (0.3–9 mg), injected into the internal maxillary artery, decreased cerebrovascular resistance in a dose-dependent manner, and this decrease was similar before and after L-NAME. Therefore, insulin-induced hypoglycemia may produce cerebral vasodilatation by releasing nitric oxide and may diminish the capacity of the cerebral vasculature to release nitric oxide in response to acetylcholine.

Keywords: Cerebral blood flow; Cerebrovascular resistance; Cerebral vasodilatation; N^G-Nitro-L-arginine methyl ester (L-NAME); Acetylcholine

1. Introduction

Hypoglycemia is a clinical entity most often caused by insulin overdose in diabetic patients, and the majority of studies indicate that acute severe and moderate hypoglycemia increases cerebral blood flow in different animal species, including humans (Della Porta et al., 1964; Hollinger and Bryan, 1987; Sieber et al., 1989; Tallroth et al., 1992). It has been also reported that the reactivity of cerebral vasculature is altered during hypoglycemia (Kim et al., 1994; Nilsson et al., 1981; Sieber et al., 1989). We have reported elsewhere that insulin-induced hypoglycemia produces cerebral vasodilatation and diminishes cerebrovascular reactivity to constrictor and dilator vasoactive stimuli in unanesthetized animals (Gómez et al., 1992). The significance and cause(s) of these effects of hypoglycemia on the cerebral circulation remain, however, uncertain (Gómez et al., 1992; Hollinger and Bryan, 1987; Pelligrino et al., 1982; Ruth et al., 1993).

Several data suggest that nitric oxide produces a basal vasodilator tone and mediates vasodilatation in response to several types of vasoactive stimuli in the cerebral circulation, indicating that cerebral blood vessels may be regulated by nitric oxide (for references, see Faraci and Brian, 1994). Nitric oxide is synthesized from L-arginine by nitric oxide synthase, and this synthesis can be inhibited by L-arginine analogues (Faraci and Brian, 1994; Moncada et al., 1991).

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Preliminary experiments from our laboratory performed in awake animals suggest that nitric oxide may mediate the cerebral vasodilatation in response to insulin-induced hypoglycemia (Fernández et al., 1993b). Results obtained by others in anesthetized piglets favor this suggestion (Ichord et al., 1994). Studies performed to examine cerebrovascular reactivity during hypoglycemia after inhibition of nitric oxide synthesis, however, have not been addressed, to our knowledge. The present study was conducted to extend our preliminary observations about the possible role of nitric oxide as a mediator of cerebral vasodilatation during hypoglycemia, and also to explore the response of the cerebral vasculature to vasodilator stimuli (acetylcholine, isoproterenol and diazoxide) during severe hypoglycemia, before and after treatment with the inhibitor of nitric oxide synthesis N^G-nitro-L-arginine methyl ester (L-NAME). The experiments were performed in awake goats and an experimental set-up was used to measure blood flow to one brain hemisphere (Reimann et al., 1972). Using this model, we have described that insulin-induced hypoglycemia produces cerebral vasodilatation (Gómez et al., 1992) and that nitric oxide may be involved in the regulation of the cerebral circulation (Fernández et al., 1993a).

2. Materials and methods

2.1. Experimental preparation

In this study 10 female goats (37–51 kg) were used. In this species, each internal maxillary artery, a branch of the external carotid artery, provides the total blood flow to each cerebral hemisphere via the rete mirabile. The vertebral arteries do not contribute to brain blood flow and the extracranial internal carotid artery is absent (Daniel et al., 1953; Reimann et al., 1972). The circle of Willis in the goat is similar to that of humans except that the blood flows in a caudal direction in the basilar artery (Daniel et al., 1953; Reimann et al., 1972). Analysis of the distribution of radioactively labeled microspheres in the cerebral circulation of the goat after the surgical procedure described by Reimann et al. (1972) indicates that nearly all of the blood carried by the internal maxillary artery passes directly to cerebral tissue (Miletich et al., 1975). Extracerebral blood flow is minimal, less than 5% of total flow.

The operative procedure, performed in anesthetized animals, has been described elsewhere (Reimann et al., 1972). Anesthesia was induced with an intramuscular injection of 10 mg/kg ketamine hydrochloride (Ketolar, Parke Davis, Morris Plains, NJ, USA), followed by i.v. administration of 2% thiopental sodium (Pentothal Sódico, Abbott, North Chicago, IL, USA); supplemental doses were given as necessary for maintenance of anesthesia during the surgical intervention. Briefly, the operative procedure is as follows: the extracerebral vessels from one of the internal maxillary arteries were ligated and thrombosed with thrombin (Thrombostat, Parke Davis) dissolved in 1 ml of 0.9% NaCl solution. This maneuver produces an almost immediate obliteration of the ethmoidal, ophthalmic and buccinator arteries, and thus eliminates blood flow to the eye and other facial structures. This is confirmed on recovery from surgery by the presence of ipsilateral blindness. However, obliteration of the extracerebral vessels from the internal maxillary artery does not cut off vascular supply to half of the face. The areas supplied by the ethmoidal, buccinator, dental and temporal arteries are nourished by anastomotic channels that are normally in the state of dynamic balance, but in which the direction of blood flow can be quickly changed, depending on the pressure differential from one side of the union to the other (Daniel et al., 1953; Reimann et al., 1972). There is no necrosis, and the functions related to these areas, such as eating, drinking and rumination, are preserved. Obliteration and thrombosis of the ophthalmic artery permanently cuts off the vascular supply to the ipsilateral eye. This procedure becomes necessary for the successful isolation of the cerebral circulation (Miletich et al., 1975; Reimann et al., 1972). The ipsilateral blindness that ensues does not seem to alter the normal behavior and physical condition of the animals.

An electromagnetic flow transducer (Biotronex, Silver Spring, MD, USA) was placed on the internal maxillary artery to measure blood flow to the ipsilateral cerebral hemisphere. A polyethylene catheter was inserted in the temporal artery and permanently fixed to measure arterial blood pressure with a Statham transducer, and to obtain samples of arterial blood in order to measure glycemia and arterial blood pO_2, pCO_2 and pH. A snare-type occluder was placed on the external carotid artery to obtain zero flow baseline values. The external connecting leads from the flow transducer and occluder, and the temporal artery catheter were led out subcutaneously and secured to the horn of the goat. Heart rate was measured from the arterial pressure pulse with a rate meter. Cerebral blood flow measurements were made with a Biotronex electromagnetic flowmeter (model BL-610). Cerebral blood flow, arterial blood pressure, and heart rate were recorded on a Beckman recorder. Cerebral vascular resistance was calculated as the mean arterial pressure in mmHg divided by cerebral blood flow in ml/min. The experiments on the unanesthetized animal started 2–3 days after the operative procedure, at which time the goat had fully recovered and was in a steady cardiorespiratory state. The various measurements were made with the goat in a large cage without restraints, except for a Lucite stock fitting loosely around the neck that limited forward and backward motion.

2.2. Experimental protocol

The procedure to induce hypoglycemia has been described elsewhere (Gómez et al., 1992). The 10 animals

were given food ad libitum, and in every case hypoglycemia was induced in the unanesthetized goat by i.v. administration of insulin (Velosulin, Novo Nordisk, Bagsvaerd, Denmark, 17 ± 5 U/kg). Five animals were subjected to three episodes of hypoglycemia at intervals of 4–6 days: two of these episodes were induced under control conditions, and the third episode was induced after i.v. administration of the analogue of L-arginine N^G-nitro-L-arginine methyl ester (L-NAME, Sigma, St. Louis, MO, USA). When the animals were treated with L-NAME, insulin was injected 20–30 min after the start of the administration of L-NAME. Three of these animals, during severe hypoglycemia and after treatment with L-NAME, received also 200–300 mg/kg of L-arginine by i.v. route. In five other animals, the effects of acetylcholine (0.01–1 μg), isoproterenol (0.03–3 μg) and diazoxide (0.3–9 mg) on cerebral blood flow were recorded during insulin-induced severe hypoglycemia, both before and after treatment with L-NAME in the same animal. In these particular experiments, hypoglycemia was induced under control conditions, and then, when the animals developed severe hypoglycemia, they were treated with L-NAME. The vasodilators used were tested during severe hypoglycemia, both before and after administration of L-NAME.

In every case, L-NAME was first injected as a bolus (35 mg/kg) and then was infused at a rate such that 12 mg/kg was administered during development of hypoglycemia. Acetylcholine (acetylcholine chloride, Sigma), isoproterenol (D,L-isopropyl-arterenol chlorhydrate, Sigma) and diazoxide (Hyperstat, Schering, NJ, USA) were dissolved in saline and administered directly into the internal maxillary artery in volumes < 0.5 ml.

At the end of each experiment, normoglycemia was restored by i.v. administration of glucose in 50% solution (Glucosmon, Leo, Madrid, Spain).

Hemodynamic variables were continuously recorded for 30–40 min before, throughout development of hypoglycemia (about 2.5–3 h) and for 30–40 min after administration of glucose. After insulin injection, glycemia began to decrease at 20–30 min, severe hypoglycemia was reached at 50–70 min, and the animals were maintained in a state of severe hypoglycemia for other 60–90 min. Arterial blood samples were taken every 15 min to measure glycemia, blood gases and pH. Glucose concentrations were determined by a glucose oxidative method (Dextrostix technique) (Glucometer II, Miles Laboratories, Elkhart, IN, USA). This method has a correlation coefficient of 0.96 compared with standard methods for glucose concentrations of 28–350 mg/dl and a coefficient of variation $< 3\%$ for glucose concentrations < 100 mg/dl. Arterial blood pO_2, pCO_2 and pH were measured by standard electronic methods (Radiometer model ABL 300, Copenhagen, Denmark). In this study, hypoglycemia was considered moderate when the blood glucose concentration was 40–60 mg/dl and severe hypoglycemia when it was lower than 30 mg/dl.

2.3. Data analysis

In the present study we first analyzed the changes in absolute values in hemodynamic variables and glycemia obtained in five animals during the two episodes of hypoglycemia considered as control episodes. This analysis showed that the results were not significantly different between the two episodes of hypoglycemia, as assessed using Student's *t*-test for paired data, indicating that these experiments provide reproducible results and can be indeed considered as control experiments. In consequence, the data obtained during these two episodes of hypoglycemia were grouped. To analyze the data obtained during hypoglycemia under control conditions and after treatment with L-NAME, an analysis of variance for repeated measures, followed by Dunnett's test, was applied to the changes in the hemodynamic variables recorded (in absolute values and in percentage), glycemia, and blood gases and pH, using the same animal as its own control.

An analysis of variance for repeated measures, followed by Dunnett's test, was also applied to data for the effects of L-NAME on hemodynamic variables recorded (in absolute values and in percentage), glycemic levels and blood gases and pH obtained under control conditions and during severe hypoglycemia, using the same animal as its own control.

The decreases in cerebrovascular resistance from the resting levels, taken as absolute values and as percentages, elicited by acetylcholine, isoproterenol and diazoxide were evaluated by an analysis of variance followed by Dunnett's test. This experiment was performed during the same episode of severe hypoglycemia, before and after L-NAME treatment. Then, the results obtained during these two conditions were compared by applying the Student's *t*-test for paired data.

$P < 0.05$ was considered statistically significant. Values are expressed as mean ± S.E.M.

3. Results

3.1. Effects of hypoglycemia under control conditions and after L-NAME treatment

(A) Under control conditions, insulin injection decreased glycemia progressively in all the animals, and we found that during moderate hypoglycemia (glycemia = 46 ± 2 mg/dl) the goats showed signs of excitation (tachycardia, horripilation, tremor, restlessness, chewing) and during severe hypoglycemia (glycemia = 26 ± 1 mg/dl) the animals showed signs of depression of the central nervous system (lethargy, little response to auditory or painful stimulation, obtundation, difficulty standing up). As glycemia decreased, a concomitant, significant increase in cerebral blood flow and decrease in calculated cerebrovascular resistance were also observed. During moder-

Table 1
Glycemia, hemodynamic values and arterial blood gases and pH obtained in five awake goats during insulin-induced hypoglycemia: (A) under control conditions (without treatment), and (B) after L-NAME treatment, alone and plus L-arginine

	(A)	Without treatment		(B)	After L-NAME treatment		
	Control	Moderate hypoglycemia	Severe hypoglycemia	Control	Moderate hypoglycemia	Severe hypoglycemia	+L-arginine (3 goats)
Glycemia (mg/dl)	90±7	46±2 [c]	26±1 [c]	83±6	46±3 [c]	24±2 [c]	26±1 [c]
CBF (ml/min)	64±4	81±6 [a]	94±8 [b]	40±4 [d]	39±3 [d]	41±5 [d]	80±9 [d,a]
CVR (mmHg/ml per min)	1.62±0.11	1.35±0.09 [a]	1.11±0.07 [a]	3.40±0.25 [d]	3.49±0.24 [d]	3.58±0.39 [d]	1.37±0.20 [b]
MAP (mmHg)	102±4	111±4 [a]	104±3	134±7 [d]	133±7 [d]	140±8 [d]	107±5 [b]
HR (beats/min)	73±6	100±8 [a]	85±8	53±5 [d]	60±5 [d]	65±8	73±8
pO_2 (mmHg)	84±3	88±3	85±4	87±4	90±5	88±4	90±6
pCO_2 (mmHg)	30±2	28±3	30±3	29±3	31±3	30±2	29±4
pH	7.40±0.01	7.42±0.02	7.39±0.02	7.39±0.02	7.40±0.02	7.40±0.01	7.39±0.02

Values are means±S.E.M. CBF, cerebral blood flow; CVR, cerebrovascular resistance; MAP, mean systemic arterial pressure; HR, heart rate.
[a] $P < 0.05$, compared with its control.
[b] $P < 0.01$, compared with its control.
[c] $P < 0.001$, compared with its control.
[d] $P < 0.01$ compared with its corresponding condition, but without treatment.

ate hypoglycemia cerebral blood flow increased by 25 ± 4% ($P < 0.05$), cerebrovascular resistance decreased by 18 ± 3% ($P < 0.05$), heart rate increased by 44 ± 6% ($P < 0.01$) and three of the animals had moderate hypertension. During severe hypoglycemia cerebral blood flow increased by 47 ± 6% ($P < 0.01$), cerebrovascular resistance decreased by 34 ± 4% ($P < 0.01$), and heart rate and arterial pressure did not change significantly. In the five goats, arterial pO_2, pCO_2 and pH values during development of hypoglycemia were comparable to those measured before induction of hypoglycemia.

The i.v. injection of a glucose solution normalized glycemia and reversed the above mentioned clinical and hemodynamic alterations within 20–30 min.

(B) In the five goats under control conditions, the i.v. injection of L-NAME increased mean systemic arterial pressure by 33 ± 2%, decreased heart rate by 28 ± 3% and resting cerebral blood flow by 37 ± 2%, and increased cerebrovascular resistance by 115 ± 7% (all $P < 0.01$). L-NAME administration also produced clinical impairment in all these animals, which showed moderate obtundation and were less responsive to laboratory stimuli. This treatment did not affect glycemia significantly (83 ± 6 vs. 90 ± 7 mg/dl, $P > 0.05$). After L-NAME treatment, the development of hypoglycemia was as under control conditions, the signs of excitation during moderate hypoglycemia were less evident, and the signs of depression of the central nervous system were comparable to those found during hypoglycemia in non-treated animals. In contrast to the changes that occurred under control conditions, cerebral blood flow, cerebrovascular resistance and heart rate did not change significantly during both moderate and severe hypoglycemia after L-NAME treatment. The levels of cerebral blood flow and cerebrovascular resistance reached after this treatment remained practically constant throughout the development of hypoglycemia.

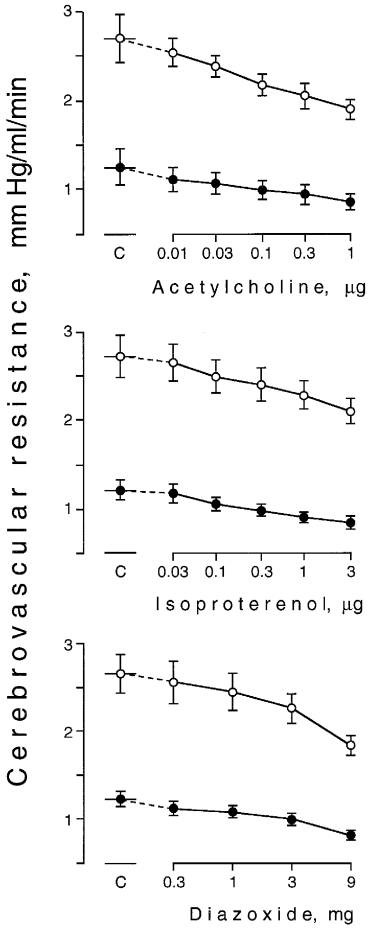

Fig. 1. Values (mean ± S.E.M.) for cerebrovascular resistance during insulin-induced severe hypoglycemia under resting conditions (control, C) and the effects of acetylcholine, isoproterenol and diazoxide obtained before (●) and after treatment with N^G-nitro-L-arginine methyl ester (L-NAME) (●) in five awake goats.

Table 2
Glycemia, hemodynamic values and blood gases and pH obtained in five goats under control conditions and during severe hypoglycemia before (alone) and after treatment with L-NAME

	Control	Severe hypoglycemia	
		Alone	+ L-NAME
Glycemia (mg/dl)	94 ± 8	27 ± 3 [a]	25 ± 2 [a]
CBF (ml/min)	58 ± 3	82 ± 5 [a]	51 ± 4 [b]
CVR (mmHg/ml per min)	1.76 ± 0.12	1.20 ± 0.08 [a]	2.70 ± 0.14 [a,b]
MAP (mmHg)	100 ± 3	99 ± 3	137 ± 5 [a,b]
HR (beats/min)	85 ± 10	109 ± 14	61 ± 8 [b]
pO_2 (mmHg)	87 ± 4	85 ± 3	85 ± 4
pCO_2 (mmHg)	31 ± 2	33 ± 3	30 ± 2
pH	7.39 ± 0.01	7.40 ± 0.02	7.40 ± 0.01

Values are means ± S.E.M. CBF, cerebral blood flow; CVR, cerebrovascular resistance; MAP, mean systemic arterial pressure; HR, heart rate.
[a] $P < 0.01$ compared with control.
[b] $P < 0.01$ compared with severe hypoglycemia alone.

In three of these animals, treatment with L-arginine at the end of hypoglycemia after L-NAME increased cerebral blood flow by 30% over the values obtained under control conditions (normoglycemia, before L-NAME treatment) and normalized arterial pressure (Table 1).

Table 1 summarizes the results obtained during hypoglycemia in the animals treated or not with L-NAME.

L-NAME by itself, and hypoglycemia before and after L-NAME treatment, did not alter significantly blood pO_2, pCO_2 and pH.

3.2. Effects of acetylcholine, isoproterenol and diazoxide during severe hypoglycemia, before and after L-NAME treatment

In five other goats, the effects of acetylcholine, isoproterenol and diazoxide were tested during the same episode of severe hypoglycemia, both before and after

L-NAME treatment. After induction of severe hypoglycemia with insulin under control conditions, cerebral blood flow increased by $42 \pm 4\%$ ($P < 0.01$), cerebrovascular resistance decreased by $31 \pm 3\%$ ($P < 0.01$), and systemic arterial pressure and heart rate did not change significantly. In these animals with severe hypoglycemia, L-NAME treatment abolished the increase in cerebral blood flow, returning it to control (normoglycemic) values, and increased significantly cerebrovascular resistance and mean systemic arterial pressure over both the preceding (hypoglycemic) and control (normoglycemic) values. Heart rate decreased with regard to the preceding (hypoglycemic) values and was similar to that recorded during control (normoglycemic) conditions (Table 2).

During severe hypoglycemia, before and after L-NAME treatment, acetylcholine (0.01–1 μg), isoproterenol (0.03–3 μg) and diazoxide (0.3–9 mg), injected into the internal maxillary artery, increased cerebral blood flow and decreased cerebrovascular resistance in a dose-dependent manner. Acetylcholine did not affect systemic hemodynamic variables. Isoproterenol increased significantly heart rate and caused slight hypotension only after administration of 3 μg, and diazoxide caused slight hypotension only after administration of 9 mg. These changes were evident after their maximal effects on cerebral blood flow.

The decreases in cerebrovascular resistance produced by acetylcholine, isoproterenol and diazoxide, both in absolute values and as percentages, from the resting resistance levels reached during severe hypoglycemia were not significantly different during severe hypoglycemia, before and after treatment with L-NAME. The absolute values for cerebrovascular resistance obtained before and after administration of acetylcholine, isoproterenol and diazoxide under the conditions tested are summarized in Fig. 1.

4. Discussion

The present data obtained during insulin-induced hypoglycemia under control conditions reproduce previous observations from our laboratory for the same experimental model (Gómez et al., 1992). They also agree with the experimental and clinical observations of others, indicating that both moderate and severe insulin-induced hypoglycemia produce cerebral vasodilatation (Della Porta et al., 1964; Hollinger and Bryan, 1987; Sieber et al., 1989; Tallroth et al., 1992), and that moderate hypoglycemia increases adrenergic activity whereas severe hypoglycemia depresses the function of the central nervous system (Gerich, 1988). We also found that the changes in cerebral blood flow after insulin-induced hypoglycemia under control conditions are reproducible when this type of hypoglycemia is repeated at least twice, suggesting that the cerebrovascular response to separate episodes of hypoglycemia under control conditions may be preserved.

With regard to mechanisms underlying cerebral vasodilatation during hypoglycemia several factors have been considered: increased arterial pressure and loss of the autoregulatory capacity of cerebral vessels (Pelligrino et al., 1982), production or release into the brain extracellular space of potassium ions (Pelligrino et al., 1982) or adenosine (Ruth et al., 1993), stimulation of β-adrenoceptors by increased plasma levels of adrenaline (Hollinger and Bryan, 1987). This particular issue, however, remains to be resolved as these factors do not provide a satisfactory explanation for the effects of different degrees of hypoglycemia on the cerebral circulation (Gómez et al., 1992).

In the present experiments, we found that L-NAME by itself decreased resting cerebral blood flow and increased cerebrovascular resistance and systemic arterial pressure. This confirms previous observations from our laboratory in awake (Fernández et al., 1993a) and anesthetized (Diéguez et al., 1993) goats, and suggests that nitric oxide produces a basal vasodilator tone in the cerebral circulation, as has been also proposed by others (for references, see Faraci and Brian, 1994). L-NAME also induced clinical alterations that might be related to a sedative effect of this substance caused by depression of the nervous system, as was previously suggested (Fernández et al., 1993a).

L-NAME did not affect the development of insulin-induced hypoglycemia, but it did modify the cerebrovascular response to this hypoglycemia. Pretreatment with L-NAME inhibited the increase in cerebral blood flow during both moderate and severe hypoglycemia, and this inhibitory effect of L-NAME was reversed by L-arginine, the substrate for nitric oxide synthesis. Thus, previous inhibition of nitric oxide synthesis blunts the development of cerebral vasodilatation in response to hypoglycemia, thus confirming preliminary results from our laboratory (Fernández et al., 1993b). In the present study we also observed that L-NAME, administered during insulin-induced severe hypoglycemia, abolished the cerebral hyperemia caused by this hypoglycemia. These later results suggest that the cerebral vasodilatation in response to hypoglycemia disappears when the synthesis of nitric oxide is inhibited. As we have previously found that the cerebral vasculature, after L-NAME administration, preserves its capacity to dilate in response to vasodilator stimuli such as diazoxide and sodium nitroprusside (Fernández et al., 1993a), we suggest that the blocking action of L-NAME on the cerebrovascular dilatation in response to hypoglycemia is not because this vasculature cannot respond to vasodilator stimuli, but is probably because L-NAME specifically inhibits mechanisms involved in the cerebrovascular response to hypoglycemia. Therefore, it is suggested that nitric oxide may be involved in the cerebral vasodilatation that occurs during insulin-induced hypoglycemia under our experimental conditions.

It has been reported that insulin produces peripheral vasodilatation mediated by nitric oxide release in humans (Scherrer et al., 1994). The possibility that the observed cerebral vasodilatation after insulin-induced hypoglycemia

is caused by the action of insulin on the cerebral vasculature rather than by the hypoglycemic state is unlikely as we found that insulin (2–8 U, three goats), injected into the internal maxillary artery, did not affect resting cerebral blood flow (data are not shown), and that normalization of glycemia by glucose injection after insulin-induced hypoglycemia restored cerebral blood flow to control values.

Our data agree with those of studies with anesthetized piglets where it has been also shown that L-NAME attenuates the vasodilatation in several regions of the central nervous system during profound insulin-induced hypoglycemia (Ichord et al., 1994). In this study (Ichord et al., 1994) L-NAME also inhibited nitric oxide synthase activity, mildly diminished the amplitude and frequency in the electroencephalogram under control conditions, and attenuated the increase in cerebral O_2 consumption during profound insulin-induced hypoglycemia. Our experimental procedure permitted us to record the evolution of the changes in cerebral blood flow during the development of hypoglycemia.

Data from the literature show that L-arginine analogues seem to inhibit both the constitutive and inducible isoforms of nitric oxide synthase, and that induction of inducible nitric oxide synthase is time-dependent and requires a relatively long period (> 2 h) of exposure to appropriate stimuli before the function of nitric oxide is expressed (for references, see Faraci and Brian, 1994; Moncada et al., 1991). In our study, the increases in cerebral blood flow were present at moderate and severe levels of hypoglycemia, and after a relatively short period of hypoglycemia, and these increases were totally blocked by L-NAME. Thus, it is suggested that the constitutive rather than the inducible nitric oxide synthase might be involved in the increased nitric oxide production during hypoglycemia under our experimental conditions. Consequently, the cerebral vasodilatation in response to insulin-induced hypoglycemia might be mainly a physiological, homeostatic response to provide glucose for the brain (Gómez et al., 1992). This study does not exclude the possibility that more severe levels of hypoglycemia acting for longer periods than in our experiments may induce the inducible nitric oxide synthase and contribute to increased nitric oxide production. Also, the present data do not exclude that other factors different from nitric oxide are involved in the cerebral vasodilatation in response to insulin-induced hypoglycemia (Hollinger and Bryan, 1987; Pelligrino et al., 1982; Ruth et al., 1993).

With regard to cerebrovascular reactivity, in a previous study from our laboratory (Gómez et al., 1992) we found that severe hypoglycemia induces a non-specific diminution of the cerebrovascular response to constrictor and dilator stimuli, which may be related, in part, to a reduced autoregulatory capacity of cerebral vessels (Pelligrino et al., 1982; Siesjö et al., 1983) and to the increased adrenergic activity (Gómez et al., 1992) that occurs during hypoglycemia. In evaluating the effects of the vasodilators acetylcholine, isoproterenol and diazoxide on the cerebral circulation before and after L-NAME administration, the fact that basal hemodynamic conditions before and after L-NAME are different should be taken into account as this might influence the subsequent responses to vasodilators. We evaluated the effects of acetylcholine, isoproterenol and diazoxide on cerebral circulation by assessing the decreases in cerebrovascular resistance. These changes can be evaluated by expressing the responses in absolute values relative to the preceding resting resistance level, as a percentage of the immediately preceding resting resistance level, or as the level of resistance reached after each dose of vasodilator. This later approach could be questionable as it may be reasonable to expect that the cerebrovascular resistance reached after vasodilator administration frequently remains higher after than before L-NAME, and it would not mean necessarily that vasodilator responses were lower after L-NAME. The second approach (percentage of preceding resting resistance level) could lead to underestimation of the effects of vasodilators during L-NAME administration, when vascular resistance is higher than before L-NAME administration. Of the three approaches considered, the first approach is least dependent on the preceding resting resistance level. In addition, it seems that increases in arterial pressure do not, necessarily, enhance the response to vasodilator agents (Gardiner et al., 1991). Thus, evaluation of the absolute reduction in cerebrovascular resistance from resting levels, as in Fig. 1, may be a more appropriate approach to analyze specific interactions between the effects of L-NAME and vasodilators. Using this approach, we found that during severe hypoglycemia, treatment with L-NAME did not affect the cerebral vasodilatation in response to acetylcholine, isoproterenol and diazoxide. The results for isoproterenol and diazoxide were expected as the vasodilatation in response to these substances is probably independent of nitric oxide release. Diazoxide, a benzothiadiazine, seems to produce relaxation of vascular smooth musculature through activation of ATP-sensitive K^+ channels (Standen et al., 1989). Isoproterenol, a β-adrenoceptor agonist, may also produce vasodilatation by mechanisms distinct from nitric oxide release in cerebral (Mayhan, 1994; Pelligrino et al., 1994) and non-cerebral (Bea et al., 1994) arteries. The results for acetylcholine, however, were unexpected as it is accepted that the vasodilatation in response to this substance in cerebral vasculature is, at least in part, mediated by nitric oxide release, although an endothelium-dependent hyperpolarizing factor may be also involved (for references, see Faraci, 1993; Faraci and Brian, 1994). We have reported elsewhere that under normal conditions L-NAME inhibits the cerebral vasodilatation elicited by lower doses (0.03–1 μg), but not the highest dose (3 μg) of acetylcholine used in awake goats (Fernández et al., 1993a). The observation that the response to every dose (0.03–3 μg) of acetylcholine was not affected by L-NAME during hypoglycemia indicates that a clear difference exists in the effects of

L-NAME on the cerebrovascular response to lower doses (0.03–1 μg) of acetylcholine in animals with normoglycemia (Fernández et al., 1993a) and severe hypoglycemia (present results). Therefore, it is suggested that severe hypoglycemia, by stimulating the release of nitric oxide, may diminish the amount of nitric oxide available for release in response to acetylcholine. This is the novelty of our study, and it might explain, at least in part, the decrease in cerebral vasodilatation in response to acetylcholine (Gómez et al., 1992), and why the cerebrovascular effects of this vasodilator were similar before and after L-NAME (present study) during severe hypoglycemia. The cerebral vasodilatation in response to acetylcholine that persists after L-NAME may be mediated by substances different from nitric oxide, such as an endothelium-dependent hyperpolarizing factor (for references, see Faraci, 1993). Therefore, severe hypoglycemia may diminish the capacity of the cerebral vasculature to release nitric oxide in response to vasodilator agents such as acetylcholine. However, on the basis that severe hypoglycemia decreases cerebrovascular reactivity to vasodilators (Gómez et al., 1992), our present results for isoproterenol and diazoxide suggest that inhibition of nitric oxide production by L-NAME does not reverse the diminution of the cerebrovascular response to dilators caused by hypoglycemia. During treatment with L-NAME the vasodilatation in response to hypoglycemia was abolished, thus the cerebrovascular bed should be able to dilate to a greater extent than during hypoglycemia without treatment with L-NAME. As this did not occur, it is suggested that inhibition of nitric oxide production does not improve the decreased cerebrovascular reactivity caused by hypoglycemia. As hypothesis, an explanation for the decreased cerebrovascular reactivity during severe hypoglycemia is that this condition might cause local metabolic alterations in cerebral perivascular regions as a consequence of the glucose deficit, which would affect vascular reactivity in a non-specific manner. The absence of glucose by itself seems to have little effect on the response of isolated vessels to vasoactive agents (Vinall and Simeone, 1986).

In conclusion, the present results suggest that hypoglycemia induced by insulin: (1) produces cerebral vasodilatation, at least in part, by releasing nitric oxide and (2) diminishes the capacity of the cerebral vasculature to release nitric oxide in response to acetylcholine. It is also suggested that inhibition of nitric oxide synthesis may not modify the effects of insulin-induced hypoglycemia on cerebrovascular reactivity to vasodilator agents.

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