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Participation of cyclooxygenase pathway in the vasoconstriction induced by 5-HT in the *in situ* autoperfused kidney of long-term diabetic rats

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ABSTRACT

We attempted to characterize the 5-hydroxytryptamine (5-HT) receptor type/subtype and mediator mechanisms involved in the contractile effects of 5-HT in the *in situ* autoperfused kidney of long-term diabetic rats. Diabetes was induced in male Wistar rats by a single subcutaneous injection of alloxan. Intra-arterial (i.a.) bolus injection of 5-HT (0.00000125 to 0.1 μg/kg) increased renal perfusion pressure in a dose dependent way but did not affect the systemic blood pressure in long-term diabetic rats. The selective 5-HT_2 receptor agonist, α-methyl-5-HT, caused a local vasoconstrictor effect in the *in situ* autoperfused rat kidney similar to 5-HT. However, BW723C86, a selective 5-HT_{2B} receptor agonist, m-CPP (1-(3-chlorophenyl)piperazine), a selective 5-$HT_{2B/2C}$ receptor agonist, the 5-HT_1 receptor agonist, 5-carboxamidotryptamine (5-CT), and the selective 5-HT_3 receptor agonist, 1-phenylbiguanide did not modify the renal perfusion pressure. In long-term diabetic rats, vasoconstriction elicited by 5-HT and α-methyl-5-HT was significantly decreased by ritanserin (a 5-HT_2 receptor antagonist), spiperone (a 5-HT_{2A} receptor antagonist), and the cyclooxygenase (COX) inhibitors, indomethacin (non-selective COX inhibitor), FR 122047 or nimesulide (selective COX-1 and COX-2 inhibitors, respectively), but was not modified by pretreatment with SB 206553 (3,5-dihydro-5-methyl-N-3-pyridinylbenzo[1,2.b:4,5-b′] dipyrrole(1H)-carboxamide hydrochloride), a non-selective 5-HT_{2C} receptor antagonist, prazosin, propranolol, enalapril or losartan. The results of protein expression support these results: COX-1 and COX-2 are expressed in renal tissue. Inducible COX (COX-2) is overexpressed in long-term diabetes. Our data suggest that, in the *in-situ* autoperfused kidney of long-term diabetic rats, 5-HT vasoconstrictor action is mediated, through cyclooxygenase pathway, by local activation of 5-HT_{2A} receptors.

1. Introduction

Diabetes and hypertension are both associated with an increased risk of renal disease. 5-Hydroxytryptamine (5-HT) affects renal function (Shoji et al., 1989; Morán et al., 1997, 2008, 2009; Tian et al., 2002); however, the action of this amine in renal vasculature is controversial regarding both the effect (vasoconstriction/vasodilatation) and the magnitude of the response. Studies performed previously by us to evaluate 5-HT-induced hemodynamic changes in several autoperfused rat vascular beds confirmed the variability of these actions depending on the vascular bed analyzed (Fernández et al., 2000; Calama et al., 2002; Morán et al., 1997, 2008, 2009). Even, in the same vascular bed, 5-HT effects depend on several factors, such as the doses used or the existence of pathologies like hypertension or diabetes (Calama et al., 2003, 2004, 2005; García et al., 2005, 2006; Morán et al., 2008, 2009, 2010).

During diabetes, 5-HT vasoconstrictor effect is enhanced in renal, pulmonary or coronary arteries of rabbits and pigs (Miranda et al., 2002; El-Kashef, 1996; Miranda et al., 2000; Bagwell and Brophy, 2000), but variable results were found in aortas of diabetic rats (Orie et al., 1993; Sikorski et al., 1993; James et al., 1994; Hattori et al., 1995).

There is evidence that increases in 5-HT plasma levels may be related to the development of diabetic nephropathy through 5-HT_{2A} receptor activation in mesangial cells (Eto et al., 1997; Kasho et al., 1998); moreover, serum concentrations of serotonin are elevated in rabbit renal artery during diabetes (Miranda et al., 2002).

It seems likely that, as previously shown by us in pithed rats (García et al., 2006), 5-HT vasoconstrictor action in renal vascular bed during diabetes is linked to changes in intracellular signaling pathways. Nevertheless, the exact mechanism has not yet been elucidated.

In Krebs-perfused diabetic rat kidneys, the activation of vascular TXA_2 receptors increases 5-HT vasoconstrictor effect to a greater

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extent than in control kidneys (Hodgson et al., 1990). Others, however, describe abnormalities in the cascade of COX in several pathologies, such as diabetic nephropathy or renal hypertension (James and Hodgson, 1995; Hao and Breyer, 2008).

In streptozotocin-induced type 1 diabetic rats, renal synthesis of prostanoids is increased (Craven et al., 1987; Hao and Breyer, 2008). COX-2 expression is also increased in the thick ascending limbs and macula densa in both type I streptozotocin diabetic and type II diabetic Zucker rats (Komers et al., 2001, 2005; Cheng et al., 2002; Dey et al., 2004). Selective COX-2 inhibition significantly reduces glomerular hyperfiltration in streptozotocin-induced diabetic rats, which is consistent with the fact that COX-2-derived prostanoids increase hyperfiltration in diabetic kidney (Komers et al., 2001, 2005; Hao and Breyer, 2008; Yar et al., 2010).

In light of the above, in this work we aimed to determine if long-term diabetic state induces changes in the 5-HT receptor type/subtype involved in the 5-HT local vasoconstrictor effect in the *in-situ* autoperfused rat kidney and analyze the possible involvement of direct/indirect mechanisms.

2. Materials and methods

2.1. Ethical approval of the study protocol

Housing conditions and experimental procedures were in accordance with regulations provided by the European Union on the use of animals for scientific purposes (86/609/EEC, Article 5; Appendix II). This was enacted by Spanish legislation on 14 March 1988 (R.D. 223) and 10 October 2005 (R.D.1201).

Male Wistar rats (250–350 g) were used in the present study. Rats were kept and supplied by the Animal House of the Faculty of Pharmacy of the University of Salamanca (PAE-SA001; Salamanca, Spain).

2.2. Diabetes induction and animal maintenance

The rats were divided into two groups: normoglycemic and diabetic rats. Diabetes was induced by a single injection of alloxan (150 mg/kg, s.c.) in 0.9% NaCl (physiological saline). Rats were then maintained on tap water and regular food *ad libitum* for 8 weeks. Normal rats served as controls, and both groups, control and alloxan-diabetic rats, were all aged-matched. Body weight, systolic blood pressure, heart rate and blood glucose levels were determined before and at 2, 7, 14, 21, 28, 35, 42, 49 and 56 days after alloxan administration. Only rats with elevated blood glucose levels (>11 mM) at all time points were considered diabetic. Blood glucose levels were determined by test strips (Accu-Chek®). Systolic blood pressure and heart rate were measured in awake rat periodical using the tail-cuff method with a photoelectric sensor (NIPREM 546, Cibertec S.A, Madrid, Spain) along the study. Several determinations were made in each session for each animal and values were considered valid if five consecutive measurements did not differ by 10 mm Hg.

2.3. Animal preparation

Diabetic and normoglycemic rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). After the induction of anesthesia, a tracheotomy was carried out and catheters placed in the right and left carotid arteries. The right carotid artery was cannulated for measurement of blood pressure using a Spectramed model P23xL pressure transducer and Grass model 7 Physiograph recorders. The penis vein was cannulated for drug administration. Rats were kept warm with a heating lamp.

Rats were prepared for *in-situ* perfusion of the left kidney according to the method of Fink and Brody (1978). Vascular beds were perfused using an extracorporeal circuit and a constant-flow Gilson peristaltic pump. The left carotid artery was cannulated with the inflow end of the extracorporeal flow line. The abdominal aorta was exposed by a midline laparotomy and deflection of the intestines to the right side of the rat. A loose tie was placed around the aorta between the left renal artery and the origin of the right renal and superior mesenteric arteries. Additional ties were placed around the aorta 1 cm below the left renal artery and 1 cm above the iliac bifurcation. Heparin sodium (5 mg/kg, i.v.) was given. An intravenous infusion of normal (0.9%) saline was initiated at a rate of 2 ml/h and continued throughout the experiment.

When the aortic tie above the left renal artery was tightened, blood immediately began to flow from the carotid artery to the left renal artery; the circuit was established without interruption of blood flow to the kidney. Blood was pumped from the right carotid artery to an aortic pouch from which the left renal artery was the only outlet (Fink and Brody, 1978; Dupont et al., 1986; Roquebert et al., 1992; Morán et al., 2008, 2009).

The distal portion of the external circuit was connected to a Spectramed model P23xL pressure transducer for measurement of perfusion pressure, which was recorded on a Grass model 7 Physiograph recorder.

At the beginning of each experiment, flow was adjusted to render the perfusion pressure equal to the systemic pressure. Flow was kept constant throughout the experiment and changes in perfusion pressure reflected changes in vascular resistance. The flow rate through the renal vascular bed ranged from 2 ml/min to 2.9 ml/min (Roquebert et al., 1992; Morán et al., 1997, 2008, 2009). In all experiments, atropine (1 mg/kg, i.v.) was administered before saline infusion was started to block the cholinergic effect.

2.4. Experimental protocols

Experiments were carried out after a 15 min period to allow blood pressure and perfusion pressure to stabilize. Five rats were used to evaluate each dose of agonist or antagonist, and each animal preparation to evaluate only one agonist or antagonist.

The first group of experiments was carried out to confirm results from our laboratory (Morán et al., 2008) in normoglycemic control rats. In this group (n = 25), 5-HT (n = 5), the selective 5-HT_1 receptor agonist, 5-carboxamidotryptamine maleate (5-CT) (n = 5), the selective 5-HT_2 receptor agonist, α-methyl-5-HT (n = 5), and the selective 5-HT_3 receptor agonist, 1-phenylbiguanide (n = 5), were administered locally at doses of 0.00000125, 0.000125, 0.00125, 0.0125, 0.025, 0.05 and 0.1 μg/kg via the distal cannula intra-arterially (i.a.) by bolus injection of a maximum volume of 10 μl using a microsyringe (Exmire microsyringe), with a gap of 5 min between administration of each drug dose. Saline solution (10 μl) was administered (i.a.) in the control group (n = 5) in the same way.

In the first alloxan-treated diabetic group (n = 35): 5-HT (n = 5), 5-CT (n = 5), α-methyl-5-HT (n = 5), the selective 5-HT_{2B} receptor agonist, α-methyl-5-(2-thienylmethoxy)-1H-indole-3-ethanamine hydrochloride (BW723C86) (n = 5), the non-selective 5-HT_{2C} receptor agonist, 1-(3-chlorophenyl) piperazine dihydrochloride (m-CPP) (n = 5), and 1-phenylbiguanide (n = 5) were administered locally at doses of 0.00000125, 0.000125, 0.00125, 0.0125, 0.025, 0.05 and 0.1 μg/kg by bolus injection (i.a.) of a maximum volume of 10 μl, with a gap of 5 min between administration of each drug dose. Saline solution (10 μl) was administered (i.a.) in the diabetic groups (n = 5) in an identical fashion.

The second alloxan-treated diabetic group (n = 30) was run in parallel with the group described above to investigate the 5-HT_2 receptor subtype involved in 5-HT renal vascular effects. Several 5-HT_2 antagonists were administered (i.v.) 10 min before the corresponding dose–response curve of the agonist was obtained. The dose of each antagonist was selected after our previous