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Effects of "*in vivo*" administration of baclofen on rat renal tubular function

Verónica Donato [a], Gerardo Bruno Pisani [b], Laura Trumper [a,c], Liliana Alicia Monasterolo [a,d,*]

[a] Área Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, (2000) Rosario, Santa Fe, Argentina
[b] Área Morfología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, (2000) Rosario, Santa Fe, Argentina
[c] Consejo de Investigaciones de la Universidad Nacional de Rosario, Argentina
[d] Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina

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ABSTRACT

The effects of the *in vivo* administration of baclofen on renal tubular transport and aquaporin-2 (AQP2) expression were evaluated. In conscious animals kept in metabolic cages, baclofen (0.01–1 mg/kg, s.c.) induced a dose-dependent increment in the urine flow rate (UFR) and in sodium and potassium excretion, associated with an increased osmolal clearance (Closm), a diminished urine to plasma osmolality ratio (Uosm/Posm) and a decrease in AQP2 expression. The above mentioned baclofen effects on functional parameters were corroborated by using conventional renal clearance techniques. Additionally, this model allowed the detection of a diminution in glucose reabsorption. Some experiments were performed with water-deprived or desmopressin-treated rats kept in metabolic cages. Either water deprivation or desmopressin treatment decreased the UFR and increased the Uosm/Posm. Baclofen did not change the Uosm/Posm or AQP2 expression in desmopressin-treated rats; but it increased the UFR and diminished the Uosm/Posm and AQP2 expression in water-deprived animals. These results indicate that *in vivo* administration of baclofen promotes alterations in proximal tubular transport, since glucose reabsorption was decreased. The distal tubular function was also affected. The increased Closm indicates an alteration in solute reabsorption at the ascending limb of the Henle's loop. The decreased Uosm/Posm and AQP2 expression in controls and in water-deprived, but not in desmopressin-treated rats, lead us to speculate that some effect of baclofen on endogenous vasopressin availability could be responsible for the impaired urine concentrating ability, more than any disturbance in the responsiveness of the renal cells to the hormone.

1. Introduction

γ-aminobutyric acid (GABA) exerts its effects through pharmacologically distinct receptors subtypes: ionotropic $GABA_A$ (Johnston, 1996) and metabotropic $GABA_B$ (Misgeld et al., 1995) receptors. Besides their abundance in central nervous system, both receptors are widely distributed in peripheral organs (Erdö, 1985; Watanabe et al., 2002). Considerable evidence has been accumulated to support the notion that GABA-ergic system plays a major physiological role outside the mammalian central nervous system (Erdö, 1985; Erdö and Wolff, 1990; Watanabe et al., 2002; Magnaghi et al., 2006; Gladkevich et al., 2006).

The $GABA_B$ agonist baclofen is extensively used as a centrally active muscle relaxant, but it has been shown clinically to exert additional effects, including analgesia, suppression of drug addiction, and cessation of chronic cough (Bowery, 2007). Moreover, actions produced by $GABA_B$-receptor activation in peripheral organs indicate other potential applications for this kind of drugs, such as asthma (Chapman et al., 1993; Tohda et al., 1998) and gastroesophageal reflux disease (Boeckxstaens et al., 2011) treatment. On the other hand, baclofen affects insulin secretion in pancreatic β-cells (Gu et al., 1993) and alters glucose homeostasis, alerting to the need to evaluate glucose metabolism during the clinical use of GABA-related drugs (Bonaventura et al., 2012). These findings encourage further investigations concerning GABA agonists and antagonists-mediated peripheral effects, in order to find new targets for therapeutic strategies or detect potential side effects during the clinical use of these drugs.

Different elements of the GABA-ergic system in the mammalian kidney were identified. GABA-like immunoreactivity was detected in epithelial cells of the ascending limbs of the Henle's loop,

* Corrresponding author at: Área Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, (2000) Rosario, Santa Fe, Argentina. Tel.: +54 341 439 3400.
E-mail addresses: lmonaste@fbioyf.unr.edu.ar, l_monasterolo@yahoo.com (L.A. Monasterolo).

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the connecting tubules, and the collecting ducts (Párducz et al., 1992). GABA release from renal tissue slices was also reported (Erdö et al., 1991). Uptake systems for GABA in apical membrane vesicles from rat kidney were described (Sidhu and Wood, 1989). GABA related enzymes, GABA-transaminase and glutamate decarboxylase, were identified in tubular cell fractions (Erdö and Wolff, 1990). Specific binding sites for the $GABA_A$ agonist, [^{3}H] muscimol (Amenta et al., 1988), and the $GABA_B$ agonist, [^{3}H] baclofen (Erdö, 1990), were found in rat kidney. These findings indicate the presence of a non-neuronal GABA system in the kidney; and the specific distribution of GABA in the tubular epithelium suggests a functional significance for this amino acid in tubular transport processes (Párducz et al., 1992). Previous experiments from our laboratory, carried out with the isolated perfused kidney model, demonstrated direct effects of GABA agonists on water and solute tubular transport (Monasterolo et al., 1996). Since the high renal perfusate flow in the isolated organ severely alters distal nephron function (Maack, 1980), we concluded that baclofen would exert these effects by acting on proximal segments.

The aim of the present study was to investigate the effects of baclofen on renal tubular function in *in vivo* models, which more closely approximate clinical situations. Physiological and pharmacological tools to evaluate baclofen effects on urinary concentrating ability were employed.

2. Materials and methods

2.1. Animals

Male Wistar rats (350–400 g body weight) were used. Animals were housed in rooms with controlled temperature (21–23 °C), and regular light cycles (12 h). They were allowed free access to a standard diet and tap water until used. Experiments were performed in accordance with the guide for the care and use of laboratory animals promulgated by the National Institute of Health (National Center for Research Resources, Bethesda, MD, USA) and approved by our institutional ethics committee.

2.2. Functional studies

2.2.1. Metabolic cage studies

Before initiation of the experiments, animals were kept in individual metabolic cages (Nalge Company, USA) during a 2-day acclimation period. Rats received a single dose of baclofen (Sigma Chemical Co., USA) of 0.01, 0.1 or 1 mg/kg body weight, s.c. at 0.15 ml/kg body weight in saline. A group of animals received the corresponding volume of baclofen vehicle (control). The doses of baclofen were chosen based on previous studies (Goto et al., 1985; Ebenezer and Pringle, 1992). Immediately after injection (17:00), animals were kept in the metabolic cages and were fasted for 16 h. Free access to water was allowed overall the experiment. At the end of the 16 h- collection period (09:00), animals were anesthetized with sodium thiopental (70 mg/kg body weight i.p.), and blood was collected from inferior vena cava into a syringe containing heparin. Creatinine, glucose, sodium and potassium concentrations and osmolality were measured in plasma and urine samples. In some experiments, excised kidneys were fixed in formalin and processed routinely according to the standard histologic procedures. Hematoxylin and eosin or periodic acid–Schiff staining techniques were performed.

In other set of experiments the effects of 1 mg/kg body weight baclofen were assayed in water-deprived rats and in rats treated with the vasopressin analog, desmopressin. In both cases, the same experimental design as described above was followed, but animals were kept in the metabolic cages with no access to water. In the first group, the effects of baclofen in 24-h water-deprived rats were tested. In the other group, rats were injected with desmopressin acetate (Dominguez Lab., Argentina) 0.1 μg/100 g body weight, s.c., one hour after the administration of baclofen. This dose of desmopressin was previously used in our laboratory (Monasterolo and Elías, 1993).

2.2.2. Conventional renal clearance studies

Rats were fasted for 16 h (17:00 to 09:00) but had free access to water before the experiments. Animals were anesthetized with sodium thiopental (70 mg/kg b body weight i.p.). The femoral vein and femoral artery were cannulated and a bladder catheter was inserted through a suprapubic incision. In these experiments, the dose of 1 mg/kg baclofen was assayed. Immediately after baclofen injection, the i.v. infusion at 2 ml/h of a saline solution containing inulin (9 mg/ml) was begun, employing a constant infusion pump (Harvard Apparatus Inc, USA). After equilibration for 60 min, urine samples were obtained during two periods of 45 min. Blood samples from the femoral artery were obtained at the midpoint of each collection period. Inulin, glucose, sodium, and potassium concentration and osmolality were measured in plasma and urine samples.

2.2.3. Measurement of biochemical parameters

Blood samples were centrifuged and plasma was immediately separated. Urine volume was measured gravimetrically. Plasma and urine inulin concentrations were determined by Roe's procedure (Roe et al., 1949). Creatinine and glucose concentrations were measured as indicated by the commercial kits (Wiener Lab., Argentina). Sodium and potassium concentrations were measured in plasma and urine samples by flame photometry, and osmolality in the same samples by using a freezing point osmometer (Osmomat 030, Gonotec GmbH, Germany). Glomerular filtration rate (GFR) was estimated by inulin or creatinine clearance, as indicated in each case. Urine flow rate (UFR), osmolal clearance (Closm) and fractional excretion of glucose (FEglu), sodium (FENa), and potassium (FEK) were calculated by conventional formulae for each animal. Urine to plasma osmolality ratio (Uosm/Posm) was also calculated.

2.3. AQP2 immunoblotting studies

Plasma membrane fractions were obtained as previously described by other authors (Marples et al., 1995). Once excised, kidneys were decapsulated. All subsequent steps were performed at 4 °C. Medullas were dissected, minced finely and homogenized in dissecting buffer (0.3 M sucrose, 25 mM imidazole, 1 mM ethylene diamine tetraacetic acid, 8.5 μM leupeptin, 1 mM phenylmethylsulfonyl fluoride, pH 7.2) in a motor-driven Teflon-glass Potter homogenizer. This homogenate was centrifuged at 4000 × *g* for 15 min. The supernatant was collected. The pellet was rehomogenized in fresh buffer and the centrifugation repeated as described above. The supernatants were pooled and centrifuged at 17,000 × *g* for 30 min. The pellet, representing the fraction enriched for plasma membrane, was resuspended in dissecting buffer and assayed for protein concentration using the method of Sedmak and Grossberg (1971). The plasma membrane fraction enrichment was confirmed by the detection of a 12-fold increase in 5′nucleotidase activity (commercial kit, Wiener Lab., Argentina), as compared with medulla homogenate samples (plasma membrane fraction = 4.88 ± 0.10, homogenate = 0.39 ± 0.04 U/l mg protein). For AQP2 detection, plasma membrane fraction samples (15 μg of protein for each sample) were subjected to sodium dodecyl sulfate- polyacrylamide gel electrophoresis (Laemmli,

1970). The polyacrylamide concentration was 8%. The amount of protein was chosen after the linearity of detection had been verified. Samples were boiled 3 min in presence of 5% 2-mercapto ethanol and 1% sodium dodecyl sulfate. The separated proteins were transferred to nitrocellulose membranes (BioRad, Hercules, CA) in Tris–glycine transfer buffer with 20% methanol in a mini-blotter (Sigma-Aldrich) (Towbin et al., 1979). Uniform blotting across the gel was verified by Coomassie brilliant blue staining of the post blot gel. Membranes were rinsed briefly in distilled water and incubated in Ponceau S solution, followed by a brief rinse in distilled water; then, they were scanned (HP ScanJet 3400C) for posterior densitometric quantitation of the Ponceau S signal. After that, the membranes were rinsed once more in distilled water until the staining was completely eliminated. The blocking step was performed for 1 h at room temperature, with 3% non-fat dry milk and 0.1% Tween, in phosphate buffer saline, followed by overnight incubation with rabbit anti-rat AQP2 immunoglobulin G (Alpha Diagnostic, Intl. Inc., USA, AQP21-A) (1:200 dilution). After washing, membranes were incubated for 1 h with alkaline phosphatase-conjugated anti-rabbit antibody (Santa Cruz Biotechnology, Inc., sc-2382) (1:2000 dilution). Alkaline phosphatase was detected with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Promega, USA). Antibody blocking experiments, carried out in our laboratory, with rat AQP2 control peptide (Alpha Diagnostic, Intl. Inc., USA, AQP21-P) demonstrated the primary antibody specificity. The densitometric analysis of the AQP2 and Ponceau S signals was performed with Gel-Pro Analyzer software. AQP2 bands densities were referred to the Ponceau S signal present in the corresponding lane. This procedure for loading control has been recently validated, under similar conditions to those used in the present work, as an alternative to actin blotting (Romero-Calvo et al., 2010).

2.4. Statistical analysis

All results were expressed as mean ± S.E.M. Statistics was performed using the one way analysis of variance followed by *t*-Student or Newman Keuls test as adequate. The 0.05 level of probability was used as the criterion of significance in all cases.

3. Results

3.1. Functional studies

Baclofen effects were studied in rats kept in metabolic cages and in rats subjected to constant saline infusion. In either experimental model, baclofen did not change the studied plasmatic parameters from those found in control animals: Osmolality (mosm/kg) = 288.00 ± 1.64; Na^+ (meq/l) = 145.40 ± 5.45; K^+ (meq/l) = 3.02 ± 0.09; glucose (g/l) = 1.21 ± 0.04; creatinine (mg/l) = 2.46 ± 0.17.

3.1.1. Effects of different doses of baclofen in conscious rats placed in metabolic cages

Baclofen treatment evoked a dose-dependent increase in urine flow rate, FENa and FEK (Fig. 1A), associated with a raised Closm and a diminished Uosm/Posm (Fig. 1B). No difference was found in FEglu, as compared with control value (0.006 ± 0.001%). GFR, estimated as creatinine clearance, did not change with baclofen administration (control = 1.15 ± 0.13 ml/min.100 g). Water intake during the experimental period was increased in the baclofen-treated animals, though no statistically significant difference among the groups was achieved (control = 8.4 ± 1.2; baclofen 1 mg/kg = 13.5 ± 3.8 ml). Histological analysis with regard to renal corpuscles and tubules injuries, perivascular edema, medullary congestion and hemorrhage, did not record any significant change in the kidneys after administration of baclofen (data not shown).

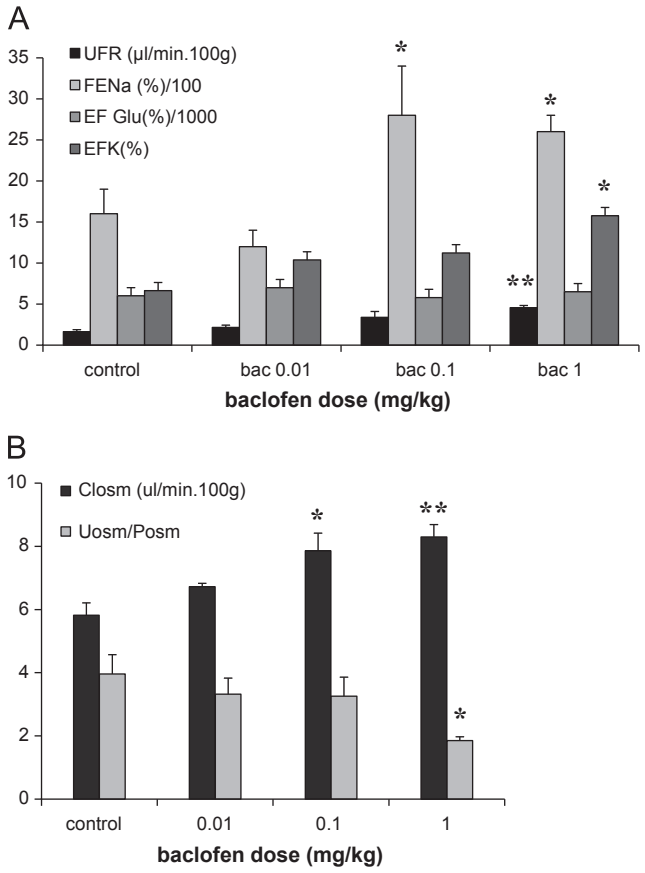

Fig. 1. *Effects of different doses of baclofen on renal function in conscious rats.* Rats received a single dose of baclofen of 0.01 ($n=4$), 0.1 ($n=5$) or 1 ($n=6$) mg/kg, body weight, or the corresponding vehicle (control, $n=5$); then, they were placed in metabolic cages with free access to water. After a 16-h urine collection period, functional parameters were calculated: (A) urine flow rate (UFR), fractional excretion of Na (FENa), glucose (FEGlu) and potassium (FEK) and (B) osmolal clearance (Closm) and urine to plasma osmolality ratio (Uosm/Posm). Data are means ± S.E.M. $^*P<0.05$ compared with control, $^{**}P<0.01$ compared with control.

3.1.2. Effects of baclofen in rats subjected to conventional renal clearance studies

Baclofen induced an increment in UFR and a diminution in sodium, potassium and glucose reabsorption. An increase in Closm associated with a diminution in Uosm/Posm was also found in baclofen-treated animals. Results are collected in Fig. 2. GFR, estimated as inulin clearance, was not altered by baclofen administration (control = 1.09 ± 0.03 ml/min.100 g).

3.1.3. Effects of baclofen in water-deprived and in desmopressin-treated rats placed in metabolic cages

The effects of baclofen on distal tubular function parameters, for water-deprived and desmopressin-treated rats, are presented in Fig. 3. Either water deprivation or desmopressin treatment provoked a decrease in UFR and an increase in Uosm/Posm, when compared with control normohydrated rats. The administration of baclofen to water- deprived rats induced a significant increment in UFR and a diminution in Uosm/Posm. On the other hand, baclofen was unable to promote changes on the mentioned tubular parameters in desmopressin-treated rats.

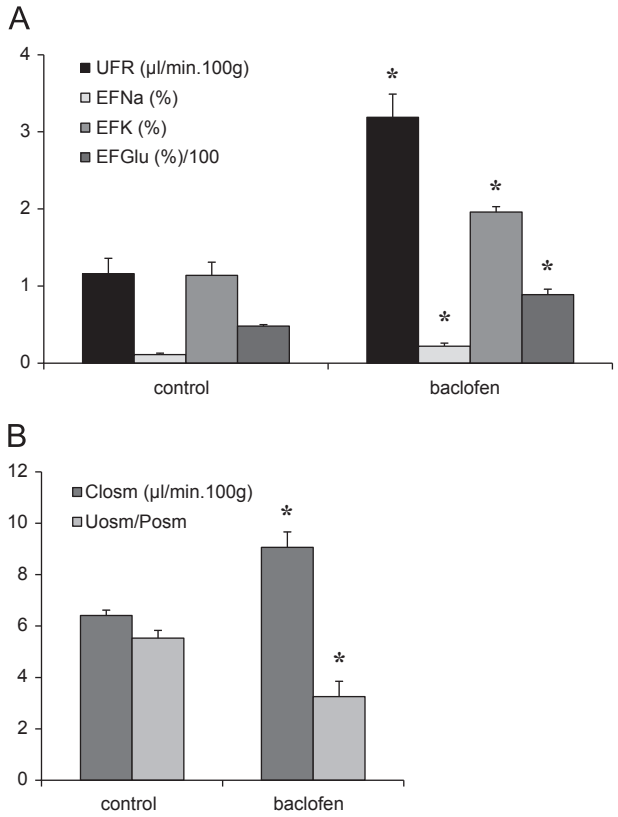

Fig. 2. *Effects of baclofen on renal function in rats subjected to clearance studies:* anesthetized rats received a single dose of baclofen of 1 mg/kg (baclofen, $n=6$), body weight, or the corresponding vehicle (control, $n=4$); then, the i.v. infusion at 2 ml/h of a saline solution was begun. After a 60-min equilibration period, two 45-min urine collection periods were carried out and functional parameters were calculated: (A) urine flow rate (UFR), fractional excretion of Na (FENa), glucose (FEGlu) and potassium (FEK) and (B) osmolal clearance (Closm) and urine to plasma osmolality ratio (Uosm/Posm). Data are means ± S.E.M. $^{*}P<0.05$ compared with control.

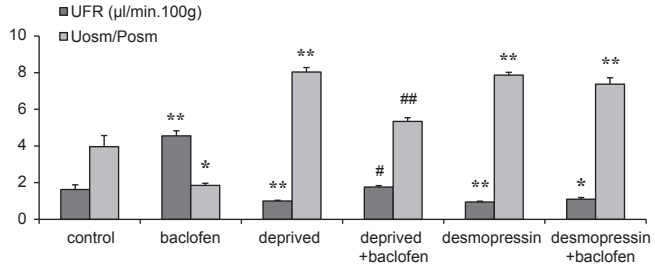

Fig. 3. *Effects of baclofen in water-deprived and in desmopressin-treated rats*: the effects of 1 mg/kg baclofen were studied in 24-h water deprived- (deprived +baclofen, $n=6$) and in desmopressin-treated (desmopressin+baclofen, $n=5$) rats. A group of 24-h water deprived- (deprived, $n=5$) and other of desmopressin-treated (desmopressin, $n=4$) rats received the corresponding vehicle. After baclofen or vehicle injection, rats were kept in metabolic cages with no access to water during a 16-h urine collection period. Urine flow rate (UFR) and urine to plasma osmolality ratio (Uosm/Posm) were calculated. Graphic also shows results from animals receiving baclofen ($n=6$) or its vehicle (control, $n=5$) in normohydration condition. Data are means ± S.E.M. $^{*}P<0.05$ compared with control, $^{**}P<0.01$ compared with control, $^{\#}P<0.05$ compared with deprived, $^{\#\#}P<0.01$ compared with deprived.

3.2. Baclofen effects on AQP2 expression

AQP2 expression was evaluated by immunoblotting in renal medullary plasma membrane fraction from rats kept in metabolic cages under different experimental conditions. Baclofen induced a decrease in AQP2 expression in renal medullary samples from normohydrated rats (Fig. 4A). Both, water deprivation (Fig. 4B) and desmopressin administration (Fig. 4C), evoked a significant increase in AQP2 expression with respect to control normohydration condition. Baclofen treatment induced a decrease in AQP2 abundance in water-deprived rats (Fig. 4B); while no change was observed in desmopressin-treated rats (Fig. 4C).

4. Discussion

The present study demonstrated that acute *in vivo* administration of the $GABA_B$ agonist, baclofen, is able to provoke renal tubular transport impairments, manifested as diuresis, natriuresis, kaliuresis, diminished urine concentrating ability and altered glucose transport. Histological analysis of renal tissue after baclofen administration did not identify any pathological alteration, indicating that the effects observed with baclofen treatment would not be consequence of cytotoxicity on renal cells.

Different experimental approaches were employed in this study. Metabolic cage experiments, carried out with conscious rats, provide the chance to evaluate renal effects of a drug a few hours after its administration, circumventing the influence of anesthetics actions or surgical stress. In this model, water intake depends on animal volition. On the other hand, conventional renal clearance techniques require the use of anesthetized animals subjected to surgical procedures. However, this model allows the possibility to obtain information about immediate renal effects, within the first minutes after the drug administration, under constant water load conditions. The results obtained from each model provide complementary information for understanding the complex *in vivo* actions of baclofen.

In the experiments carried out in conscious rats, dose-dependent effects of baclofen on urine flow rate, sodium excretion, osmolal clearance and urine osmolality-to-plasma osmolality ratio were observed. This finding adds new evidence to our previous study reporting diuresis, natriuresis and increased glucose excretion induced by baclofen in the isolated perfused rat kidney (Monasterolo et al., 1996). The isolated kidney model requires high perfusion flow rate in order to maintain the preparation under adequate oxygenation; and this fact rules out the chance to properly analyze the distal tubular function (Maack, 1980). The augmented osmolal clearance and the decreased urine osmolality observed in the present work clearly point out actions exerted on distal tubular renal structures.

The results obtained from rats kept in metabolic cages were cautiously considered, since the water intake in this experimental model depends on the animal volition. Previous studies demonstrated that baclofen affects drinking in rats under a variety of conditions, but it had no effects on water intake in normohydrated animals (Ebenezer et al., 1992; Houston et al., 2012). In our experiments, the administration of baclofen to normo- hydrated rats induced a marked trend toward an increase in water intake, as compared with non-treated rats. In spite of the fact that this increase in water intake did not achieve statistical significance, it could entail renal physiological implications. Thus, a series of experiments was performed using conventional renal clearance techniques. This protocol allows the maintenance of a constant water load. Under these experimental conditions, baclofen induced an increase in urine flow rate, fractional excretion of sodium, potassium and osmolal clearance, associated with a decrease in Uosm/Posm. These results showed that baclofen is able to provoke similar alterations on tubular renal function to those observed in conscious rats, independently of its effects on water intake.

In the continuous infusion experiments, the effects of baclofen were evaluated one hour after its administration. At this time,

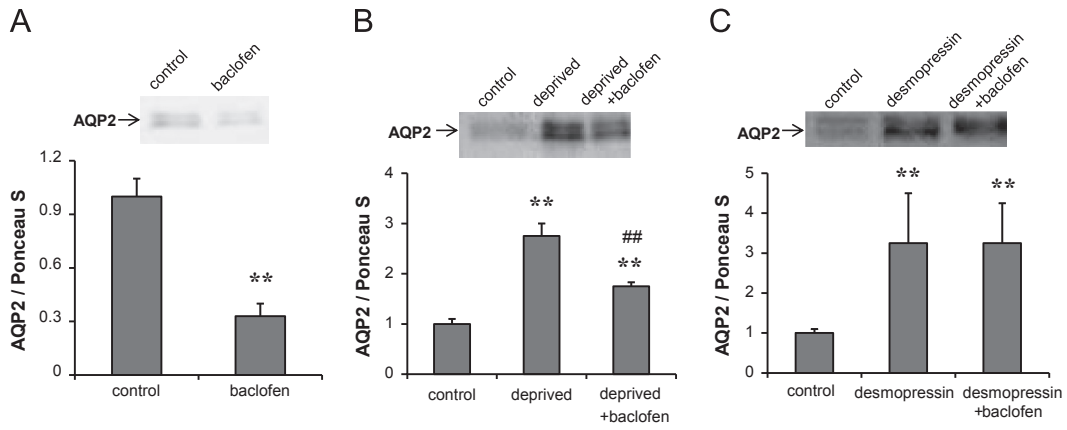

Fig. 4. *AQP2 abundance in medullary plasma membrane fractions.* Representative western blots showing AQP2 expression in samples obtained from: (A) rats receiving baclofen (baclofen, $n=6$) or its vehicle (control, $n=5$) in normohydration condition, (B) control ($n=5$), water-deprived (deprived, $n=5$) and water-deprived receiving baclofen (deprived+baclofen, $n=6$) rats, (C) control ($n=5$), desmopressin-treated (desmopressin, $n=4$) and desmopr. receiving baclofen (desmopressin+baclofen, $n=5$) rats. In each case, the results are shown as the ratio between AQP2 band density and the PonceauS signal in arbitrary units. Bars represent means ± S.E.M. $^{**}P<0.01$ compared with control, $^{\#\#}P<0.01$ compared with deprived.

a diminished glucose tubular reabsorption without changes in glucose plasma concentration was found. This impairment in renal glucose transport had been previously described by our group in the isolated rat kidney model (Monasterolo et al., 1996), indicating that the renal proximal tubule could be a target for the $GABA_B$ agonist actions. This proposal is consistent with the localization of [^{3}H]baclofen binding sites within the rat renal cortex with high specific labeling in tubular structures (Erdö, 1990). Conspicuously, this alteration in glucose tubular transport was not detected in rats kept in metabolic cages during 16 h after baclofen administration. Taken together, the findings arisen from the different experimental models suggest that baclofen alters glucose tubular transport *in vivo* by directly acting on renal structures in an immediate and transient fashion. It could be suspected that in a 16-h period some systemic action, not fully understood by now, may counteract the altered renal glucose excretion. The amounts of residual urine in the rat bladder, at the beginning and at the end point of the metabolic cages experiments, may also contribute to mask transient effects on renal function within the experimental period. Although the acute *in vivo* administration of baclofen seems to induce short-lasting effects on renal glucose transport, it would be important to investigate the renal response to repeated dose administration, when a steady-state plasma concentration of the drug is achieved. Interestingly, several authors (Gu et al., 1993; Braun et al., 2004; Brice et al., 2002; Bonaventura et al., 2012) demonstrated that acute or chronic administration of drugs acting through $GABA_B$ receptors induces alterations in glucose homeostasis by affecting pancreatic function. In recent years, increased attention has been focused on the role of kidney in glucose homeostasis (DeFronzo et al., 2012). In this connection, the present results suggest the convenience of taking into account the renal effects of $GABA_B$ related drugs in the overall comprehension of the observed glucose homeostasis alteration.

The baclofen-induced increase in osmolal clearance reflects an augmented cortical collecting duct flow rate, indicating the inefficient urine dilution as consequence of impaired electrolyte reabsorption at the thick ascending loop of Henle (Kamel et al., 1996). The administration of the $GABA_B$ agonist evoked a diminution in Uosm/Posm. This fact could be assumed as the expected decrease in the collecting tubule water reabsorption in response to the insufficiently dilute incoming fluid and the decreased interstitial tonicity. Nevertheless, other mechanisms could be contributing to the impaired urine concentrating ability induced by baclofen. Vasopressin regulates water permeability by controlling the number of AQP2 water channels in the apical membrane of collecting-duct cells (Wade et al., 1981; Harris et al., 1991; Nielsen et al., 1995). In the present study, the expression of AQP2 in plasma membrane fraction, obtained from renal medulla, was found diminished by baclofen treatment. Since the loss of the high interstitial tonicity in the inner medulla does not affect AQP2 levels (Marples et al., 1996), the decreased protein expression suggests that baclofen might exert its effects by affecting vasopressin regulation of collecting tubule water permeability. In order to further evaluate this hypothesis, experimental models of demanding collecting duct water reabsorption conditions, such as water deprivation or desmopressin administration, were employed. Both treatments induced a significant decrease in urine flow rate associated with an augmentation in Uosm/Posm and solute-free water reabsorption, with respect to control normohydrated animals. AQP2 expression in plasma membrane fraction was found enhanced in water-deprived and desmopressin- treated rats. These effects on renal function and AQP2 abundance were previously described by other authors in water-deprived and vasopressin- or its analogues-treated rats (Atherton et al., 1971; Elalouf et al., 1986; Marples et al., 1995; Bonilla-Felix and Jiang, 1997). After a 24-h water-deprivation period, a 5-fold increase in vasopressin plasma concentration in rats was reported (Dunn et al., 1973). This experimental condition constitutes a useful tool to evaluate renal effects depending on endogenous antidiuretic hormone levels. Baclofen administration did not induce alterations in the urine concentrating ability or in AQP2 expression levels in desmopressin-treated rats, suggesting that the $GABA_B$ agonist is unable to alter the renal response to the hormone. In contrast, baclofen induced an increase in urine flow rate and a decrement in Uosm/Posm, associated with a diminution in AQP2 expression in water-deprived rats. This fact leads to the hypothesis that baclofen impairs urine concentrating capacity by interfering with endogenous antidiuretic hormone availability. In this connection, evidence has been accumulated that $GABA_B$ receptors might be involved in mechanisms engaged in the regulation of vasopressin secretion in conscious rats (Yamaguchi and Yamada, 2008; Yamaguchi and Hama, 2011). Experiments to prove this hypothesis will be carried out in our future investigations.

In conclusion, this study provides evidence that *in vivo* administration of the therapeutically used $GABA_B$ agonist, baclofen, induces disturbances in renal tubular function by acting at different segments of the nephron. The impaired glucose reabsorption points out to the proximal tubule as a possible target for baclofen. The distal tubular function was also affected. The augmented osmolal clearance in baclofen- treated animals suggested a defect in electrolytes transport in the ascending limb of the loop of Henle. The diminution in urine osmolality and AQP2

expression indicates a possible impairment in vasopressin-regulated collecting duct water reabsorption. Since baclofen was able to decrease Uosm/Posm and AQP2 abundance in water-deprived rats and failed to alter the response to the exogenously administered hormone, it could be thought that mechanisms affecting endogenous vasopressin availability are involved in baclofen effects. These results reinforce the hypothesis defended by other authors (Erdö, 1990) and by ourselves, that the components of the GABAergic system present in the kidney may be involved in modulatory mechanisms of the renal function. On the other hand, the reported effects of baclofen should be taken into account in future studies in order to evaluate their clinical significance.

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