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Estrogenic regulation of NADPH-diaphorase in the supraoptic and paraventricular nuclei under acute osmotic stress

Daniela Grassi [a,b,c], Natalia Lagunas [a,b], Miguel Amorin [b], Helena Pinos [a], GianCarlo Panzica [c], Luis M. Garcia-Segura [b] and Paloma Collado [a]

a Universidad Nacional de Educacion a Distancia (UNED), Department of Psychobiology, Calle Juan del Rosal 10, 28040 Madrid, Spain

b Instituto Cajal, CSIC, Avenida Doctor Arce 37, 28002 Madrid, Spain

c University of Turin, Department of Neuroscience, Corso Massimo D'Azeglio 52, 10126 Turin, Italy and Neuroscience Institute Cavalieri Ottolenghi (NICO), Regione Gonzole 10, 10043, Orbassano, Italy

Corresponding author: Grassi D, Universidad Nacional de Educacion a Distancia (UNED), Department of Psychobiology, Calle Juan del Rosal 10, 28040 Madrid, Spain
e-mail: dada.grassi@gmail.com

Abstract

Estrogen receptors (ERs) α and β are involved in the regulation of nitrergic system in the supraoptic (SON) and paraventricular (PVN) nuclei under basal conditions. In this study we have assessed whether ERs are also involved in the modulation of the nitrergic system in the SON and PVN under acute systemic hypertonic conditions. Adult ovariectomized rats received a single injection of either estradiol, a selective ERα agonist, a selective ERβ agonist, a selective ERα antagonist, a selective ERβ antagonist or vehicle. Twenty-four hours later, animals received one i.p. injection of 1.5M NaCl to induce osmotic stress and were sacrificed after two additional hours. The number of NADPH-diaphorase positive cells in the SON and PVN was determined. Their number in the SON was not affected by NaCl administration, whereas in the four PVN subdivisions it was decreased after NaCl administration. Estradiol and the ERα agonist prevented the action of NaCl in the four subdivisions of the PVN. In contrast, the inhibition of ERα enhanced the effect of NaCl, inducing a further decrease in the number of NADPH-diaphorase positive cells. Moreover, the ERβ agonist enhanced and the ERβ antagonist blocked the effect of NaCl on the number of NADPH-diaphorase positive neurons in the SON and in the medial magnocellular subdivision of the PVN. These findings indicate that estradiol regulates the nitrergic system in the SON and PVN under acute osmotic stress conditions, but the effects specifically depend on the anatomical subregions and different ERs.

Keywords: Arginine-Vasopressin; Estradiol; Estrogen receptors; Estrogen receptor ligands; Hypothalamus; Nitric oxide; Oxytocin; Paraventricular nucleus; Supraoptic nucleus; Water balance;

1. Introduction

The supraoptic (SON) and the paraventricular hypothalamic (PVN) nuclei are involved in the regulation of different relevant physiological responses (Swanson and Sawchenko, 1980; Higuchi and Okere, 2002; Engelmann et al., 2004), having a critical role in the control of body fluid homeostasis (Mueller et al., 2006; Heesch et al., 2009). The magnocellular neurons in the SON and the PVN, responsible for the release of arginine-vasopressin and oxytocin, are sensitive to changes in intracellular Na^+ concentration and osmolarity in mammals and other vertebrates (Verney, 1958; Leng et al., 1982; Ramieri and Panzica, 1989; Voisin et al 1997).

Nitric oxide (NO) plays a relevant role in the control of fluid balance homeostasis (Calapai et al., 1992, 1994). The neural isoform of nitric oxide synthase (nNOS) enzyme, responsible for the formation of NO, has the same distribution and properties of the enzyme NADPH-diaphorase (Dawson et al., 1991). Therefore histochemistry for NADPH-diaphorase is considered a reliable method to identify the neurons that produce NO (Hope et al., 1991; Dawson et al., 1991; Vincent and Kimura, 1992; Rodrigo et al., 1994). In rodents, NADPH-diaphorase activity and nNOS immunoreactivity have been detected in magnocellular neurons of the SON and the PVN and in the circumventricular organs (CVOs), such as the subfornical organ (SFO) and the organum vasculosum laminae terminalis (OVLT), structures involved in osmoregulation (Dawson et al., 1991; Kadowaki et al., 1994; Rodrigo et al., 1994; Bhat et al., 1995; Cork et al., 1998; Liu et al., 1998; Gotti et al. 2005). Under conditions of osmotic stress (water deprivation), the number of NADPH-diaphorase cells as well as the nNOS synthase gene expression and the production of NO increase in the SON and the PVN (Kadowaki et al., 1994; Ueta et al., 1995; Srisawat et al., 2004; Gillard et al, 2007; Ryu et al., 2008).

Gonadal hormones play an important role in the regulation of the brain nitrergic system (Panzica et al., 2006). Specifically, in rodents, nNOS expression is modulated throughout the estrous cycle in brain structures involved in the control of reproductive behavior, such as the medial preoptic area, the arcuate nucleus, the ventromedial hypothalamic nucleus, the bed nucleus of the stria terminalis (Sica et al., 2009), the bed nucleus of the accessory olfactory tract (Collado et al., 2003), the anteroventral subdivision of the amygdala (Carrillo et al., 2007), as well as the hippocampus (Gotti et al, 2009). In all of these brain regions, nNOS cell number is increased when estradiol levels rise during the estrous cycle. Regulation of nitrergic system by gonadal hormones has been also demonstrated in the SON and the PVN. Estradiol, via estrogen receptors (ERs) α and β, regulates NADPH-diaphorase activity in the SON and the PVN. In the SON, the number of NADPH-diaphorase positive cells is decreased by ERα and ERβ agonists and increased by ERα and ERβ antagonists. In contrast, the activation of ERα increases the number of NADPH-diaphorase positive cells in the PVN subnuclei (parvocellular, PaV; lateral magnocellular, PaLM; medial magnocellular, PaMM; dorsal parvocellular, PaDC), while the activation of ERβ has the opposite effect (Grassi et al., 2012).

Although these findings indicate that the number of NADPH-diaphorase positive cells in the SON and the PVN is regulated by estradiol through ERα and ERβ, it is unknown whether this regulation is altered in conditions of osmotic stress. Under these conditions, magnocellular neurons suffer different structural and functional changes, including modifications in synaptic function (Kim et al., 2011), transcriptional activity (Lafarga et al., 1998; Arima et al., 1999; Berciano et al., 2002) and expression of ERs (Somponpun and Sladek, 2003, 2004). Since these modifications may alter the

response of magnocellular neurons to estradiol, it is important to determine the regulation exerted by ERα and ERβ on the nitrergic system in the SON and the PVN neurons under conditions of osmotic stress. Therefore, in the present study we have assessed the effect of different ERα and ERβ ligands on the number of NADPH-diaphorase positive cells in the SON and the PVN of adult female ovariectomized rats under acute osmotic stress.

2. Experimental procedures

2.1 Animals and experimental treatments

Wistar albino female rats from our in-house colony were kept on a 12:12-h light–dark cycle and received food and water ad libitum. Animals were handled in accordance with the guidelines published in the in accordance with the guidelines presented in the "NIH Guide for the care and use of laboratory animals", the principles presented in the "Guidelines for the Use of Animals in Neuroscience Research" by the Society for Neuroscience, and following the European Union legislation (86/609/EEC) and the Spanish Government Directive (R.D. 1201/2005). Experimental procedures were approved by our Institutional Animal Use and Care Committee. Special care was taken to minimize animal suffering and to reduce the number of animals used to the minimum necessary.

Female rats were bilaterally ovariectomized at the age of 3 months under isoflourane anaesthesia. They were then housed in plastic cages and randomly assigned to the different treatments. Seven days after surgery 4 rats per each group received one i.p. injection of vehicle (corn oil), 17β-estradiol (Sigma, Madrid, Spain; 50 μg/kg), the selective ERα agonist-PPT (4,4',4''-(4-Propyl-[1*H*]-pyrazole-1,3,5-triyl)trisphenol; BiogenCientifica, Madrid, Spain; 1 mg/kg), the selective ERβ agonist-DPN (2,3-bis(4-

hydroxyphenyl)-propionitrile; BiogenCientifica; 1 mg/kg), the selective ERα antagonist-MPP (1,3-Bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy) phenol]-1H-pyrazole dihydrochloride; BiogenCientifica; 1 mg/kg), or the selective ERβ antagonist-PHTPP (4-[2-Phenyl-5,7-*bis*(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidin-3-yl]phenol; BiogenCientifica; 1 mg/kg). Doses of ER ligands were based on previous studies in vivo (Waters et al., 2009; Santollo et al., 2010; Grassi et al., 2012).

Twenty-four hours after the injection of the estrogenic compounds or the vehicle, the animals received one i.p. injection of 1.5 M NaCl to induce salt load. Two hours after salt load induction all the animals were deeply anesthetized with pentobarbital (Normon Veterinary Division, Madrid, Spain, 50 mg/kg) and perfused through the left cardiac ventricle with 50 ml of saline solution (0.9% NaCl) followed by 250 ml of fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). Brains were quickly removed and immersed for 4–6 hours at 4ºC in the same fixative solution and then rinsed with phosphate buffer. Brains were placed for 72 hours in a 30% sucrose solution in PBS, frozen in liquid isopentane at −35ºC, and stored in a deep freezer at −80ºC until sectioning. Brains were serially cut in the coronal plane at 25 μm thickness with a cryostat. The plane of sectioning was oriented to match the drawings corresponding to the transverse sections of the rat brain atlas of Paxinos and Watson (1986). Sections were collected in a cryoprotectant solution (Watson et al., 1986) at -20°C. Every fourth section (one section every 100 μm) was histochemically stained for NADPH-diaphorase.

2.2 NADPH-diaphorase histochemistry

The presence of NADPH-diaphorase, a specific marker for nitric oxide-producing neurons (Hope et al., 1991; Dawson et al., 1991; Vincent and Kimura, 1992; Rodrigo

et al., 1994) was detected by histochemistry performed on free-floating sections. Before the reaction, the sections collected in the cryoprotectant solution were washed for 30 min at room temperature in PBS 0.01 M, pH 7.3–7.4 and then overnight at 4ºC in phosphate buffer 0.1 M, pH 7.4. The following day, free floating sections were washed at first in TRIS buffer 0.1 M, pH 8.0 for 15 min at room temperature, then in TRIS buffer, pH 8.0 containing 0.5% Triton X-100 for 10 min. The sections were incubated for 30 min at 37°C in a solution of 0.8 mM nitrobluetetrazolium (Sigma, Madrid, Spain) and 1 mM reduced NADPH (Sigma, Madrid, Spain) in TRIS buffer, 0.1 M, pH 8.0, containing 0.5% Triton X-100. The sections were washed in the same buffer and collected on chromallum coated slides, air dried, cleared in xylene, and cover slipped with Depex (VWR International Eurolab, Barcelona, Spain) for quantitative analysis.

2.3 Morphometric analysis

The quantitative analysis of NADPH-diaphorase staining was performed on coded sections without knowledge of the experimental group. All NADPH-diaphorase positive cells were considered for quantification. Selected fields were acquired by a digital camera (Olympus DP25) connected to a Nikon eclipse E600 microscope using a x20 objective. The number of NADPH-diaphorase positive cells within four coded sections per animal was studied in the SON and the PVN in the right hemisphere. Since the anatomical heterogeneity of the PVN, a grid of 24 square boxes of 8 μm^2 each were used in each slice and the grid was precisely located ensuring the left side of the grid on the wall of the third ventricle and that the grid overlap the total area of the PVN. Cell counting was performed for each square box within the boundaries of the nucleus. The PVN subdivisions were identified following the detailed anatomical description provided by Armstrong et al (1980) based on Nissl-stained material. The

boxes corresponding to the same PVN subdivision along the four coded sections were grouped for the statistical analysis (see Grassi et al., 2012, Fig. 1).

2.4 Statistical Analysis

Data were analyzed by one-way ANOVA (being the treatment the independent variable) followed by Bonferroni's post-hoc test, using the SPSS-17.0 software (SPSS Inc, Chicago, USA). A value of $P < 0.05$ was considered statistically significant. Data are represented as the mean±SEM.

3. Results

In the present study, NADPH-diaphorase positive cells in the SON and in all the PVN subdivisions (PaDC, PaLM, PaMM and PaV) have been assessed in four animals of each experimental group. NADPH-diaphorase staining was present in the SON and in all the PVN subdivisions. Qualitative observation of NADPH-diaphorase positive cells in the SON and in the different PVN subregions revealed differences in the pattern of histochemical staining among the different experimental groups. These qualitative differences were confirmed by the quantitative analysis. Representative examples of NADPH-diaphorase positive cells in the SON and the PVN are shown in figures 1 and 2.

3.1 Quantitative analysis of NADPH-diaphorase positive cells in the supraoptic nucleus (SON)

In the SON, one-way ANOVA showed a significant effect of the treatment on the number of NADPH-diaphorase positive cells [$F_{(6,25)}= 15.79$; $P<0.0001$]. Post-hoc analysis with the Bonferroni's test revealed that, compared to control values, the number of NADPH-diaphorase positive cells was not significantly affected by NaCl,

estradiol, the ERα agonist-PPT or the ERα antagonist-MPP. In contrast, the ERβ agonist-DPN induced a significant decrease in the number of NADPH-diaphorase positive cells, compared to control and NaCl values, whereas the ERβ antagonist-PHTPP had the opposite effect (Table. 1, Fig 3).

3.2 Quantitative analysis of NADPH-diaphorase positive cells in the PVN

In the PaDC, the PaLM and the PaV subdivisions, one-way ANOVA revealed a significant effect of the treatment on the number of NADPH-diaphorase positive cells [$F_{(6,29)}$=80.17, P<0.0001; $F_{(6,29)}$=242.5, P<0.0001 and $F_{(6,29)}$=57.47, P<0.0001 respectively]. Post-hoc analysis with the Bonferroni's test revealed that NaCl significantly decreased the number of NADPH-diaphorase positive cells, compared to control values. In addition, the number of NADPH-diaphorase positive cells was increased by estradiol and by the ERα agonist-PPT and was decreased by the ERα antagonist-MPP compared to control and NaCl values. In contrast, the ERβ agonist-DPN and the ERβ antagonist-PHTPP did not show significant effects on the number of NADPH-diaphorase positive cells compared to NaCl values (Table 1, Fig 4).

In the PaMM, one way ANOVA revealed a significant effect of the treatment on the number of NADPH-diaphorase positive cells [$F_{(6,29)}$= 138.6; P<0.0001]. NaCl significantly decreased the number of NADPH-diaphorase positive cells, compared to control values. Estradiol as well as the ERα agonist-PPT induced a significant increase in the number of NADPH-diaphorase positive cells compared to control and NaCl values. In contrast, the treatment with the ERα antagonist-MPP or the ERβ agonist-DPN caused a significant decrease of the number of NADPH-diaphorase positive cells compared to control and NaCl values. Treatment with the ERβ antagonist-PHTPP

caused a significant increase of the NADPH-diaphorase positive cells compared to NaCl values. (Table 1, Fig 4).

4. Discussion

Our findings indicate that the number of NADPH-diaphorase positive cells is decreased in all the subdivisions of the PVN after acute administration of 1.5 M NaCl to female rats. Since NADPH-diaphorase is considered a specific marker for nitric oxide-producing neurons (Hope et al., 1991; Dawson et al., 1991; Vincent and Kimura, 1992; Rodrigo et al., 1994), these data confirm that acute osmotic stress may have a profound effect on the NO producing system in this nucleus.

In contrast to the effect on the number of NADPH-diaphorase positive cells in the PVN, the administration of NaCl did not significantly affect this parameter in the SON. The different synaptic inputs that convey osmotic information to the SON and the PVN from the CVOs may be involved in this regional difference in the effect of acute osmotic stress. In fact, the SFO and OVLT projections to the SON are mainly GABAergic, while the projections from these CVOs to the different subregions of the PVN are glutamatergic (Pyner, 2009; Tavares et al., 2005). Therefore, the CVOs may inhibit or stimulate the nitrergic system depending on the target nucleus.

Estradiol is known to increase the responsiveness of vasopressinergic and oxytocinergic neurons to osmotic stimulation (Mecawi et al., 2011; Vilhena-Franco et al., 2011). This effect is probably not mediated by the inhibition of NO release, since estradiol is also known to increase nNOS activity (Gingerich and Krukoff, 2008) and the number of NADPH-diaphorase positive cells (Grassi et al., 2012) in the hypothalamus. Indeed, our present findings indicate that estradiol increases the number

of NADPH-diaphorase positive neurons in the PVN of NaCl treated rats, suggesting that the hormone increases the number of neurons producing NO in this nucleus under acute osmotic stress conditions. Since the PVN is an integration center for neuroendocrine and autonomic functions, estradiol may regulate a variety of homeostatic responses to cope with acute osmotic stress by the modification of NO production in this nucleus.

In a previous study we have shown that ERα and ERβ exert a differential regulation on the number of NADPH-diaphorase positive neurons in the SON and the PVN under basal conditions (Grassi et al., 2012). The activation of ERα increases the number of NADPH-diaphorase positive neurons in the PVN but decreases it in the SON. In contrast, the activation of ERβ decreases the number of NADPH-diaphorase positive neurons in both PVN and SON (Grassi et al., 2012). Our present findings indicate that ERα and ERβ also contribute to the regulation of the number of NADPH-diaphorase positive neurons in the PVN and the SON under acute osmotic stress conditions. However, acute osmotic stress caused some interesting modifications in the regulatory action of ERs. Thus, the ERβ agonist-DPN and the ERβ antagonist-PHTPP were unable to modify the number of NADPH-diaphorase positive neurons in the PaLM, PaDC and PaV subdivisions of the PVN under conditions of acute osmotic stress. However, ERβ agonist-DPN was still able to decrease the number of NADPH-diaphorase positive neurons in the PaMM and the SON. In both cases, ERβ agonist-DPN not only prevented the effect of NaCl, but even decreased the number of NADPH-diaphorase positive neurons to values significantly lower than those observed in NaCl treated. The ERβ antagonist-PHTPP had the opposite effect, enhancing the effect of NaCl.

The alteration in the action of ERβ ligands on the PaLM, PaDC and PaV may, at least in part, be related to the decrease in the expression of ERβ in PVN magnocellular neurons caused by osmotic stress (Somponpun and Sladek, 2003, 2004). This downregulation of ERβ expression may explain why ERβ ligands do not affect the number of NADPH-diaphorase positive neurons in the PaLM, PaDC and PaV subregions of the PVN under acute osmotic stress. However, the ERβ ligands were able to alter the number of NADPH-diaphorase positive neurons in the SON and the PaMM, even if osmotic stress also causes a depletion of ERβ in these structures (Somponpun and Sladek, 2003, 2004). A possible explanation for the ERβ-dependent modulation of the NADPH-diaphorase positive neurons in the SON and the PaMM under acute osmotic stress conditions is an indirect action of these ligands on SON and PaMM neuronal afferents expressing ERβ. Such afferents may originate in dorsal raphe, which neurons, in rat, predominantly express ERβ over ERα (Lu et al., 2001; Suzuki et al., 2012), project to both SON and PaMM (Conrad et al., 1974; Azmitia and Segal, 1978; Moore et al., 1978; Sawchenko et al., 1983; Tribollet and Dreifuss, 1991) and are involved in the osmotic response to hypertonic stimuli (Olivares et al., 2003; Cavalcante-Lima et al., 2005; Godino et al., 2007).

Acute osmotic stress did not affect the regulatory action of ERα on the number of NADPH-diaphorase positive neurons in the PVN. As observed for basal conditions (Grassi et al., 2012), the ERα agonist-PPT increased the number of NADPH-diaphorase positive neurons in the four subdivisions of the PVN in the rats submitted to acute osmotic stress. Activation of ERα not only prevented the effect of NaCl, but even increased the number of NADPH-diaphorase positive neurons to values significantly higher than the NaCl group. The inhibition of ERα activity with ERα antagonist-MPP had the opposite effect, enhancing the effect of NaCl. Since neurons in

the PVN express predominantly ERβ, the regulation exerted by ERα ligands on NADPH-diaphorase positive neurons in this nucleus probably reflect estrogenic actions on ERα-sensitive neurons of the OVLT projecting to the PVN (Somponpun et al., 2004; McKinley et al., 2004).

In contrast, the ERα ligands were unable to alter the number of NADPH-diaphorase positive neurons in the SON. This suggests that acute osmotic stress impairs the action of ERα ligands in the SON, since under basal conditions the ERα agonist-PPT decreases and the ERα antagonist-MPP increases the number of NADPH-diaphorase positive neurons in this nucleus (Grassi et al., 2012). Since the SON does not express ERα, the action of acute osmotic stress should affect the activity of ERα expressing afferent neurons, such as the SFO, the OVLT, the AnteroVentral Periventricular nucleus (AVPe) and the Median Preoptic Nucleus (MPO) (Gruber et al., 1986; Voisin et al., 1997; Oliveira et al., 2004; de Carvalho Borges et al., 2006; Sladek and Somponpun, 2008).

Although ERα and ERβ exert a differential regulation of the number of NADPH-diaphorase positive neurons in the SON and the PVN, the effect of estradiol is similar to the effect of ERα in both nuclei. This suggests that the mechanisms activated by the hormone that are mediated by ERα predominate over those that are mediated by ERβ. Therefore, we may speculate that the action of estradiol on the number of NADPH-diaphorase positive neurons in the SON and the PVN under acute osmotic stress conditions is mediated predominantly by neuronal afferents expressing ERα.

In the present study we have observed effects of ER antagonists on the number of NADPH-diaphorase positive neurons in the SON and the PVN of ovariectomized rats,

which are deprived of gonadal estrogens. This observation raises the important consideration on the nature of the endogenous ligands that are activating ERs under these conditions. It is known that the intracellular signaling pathways of several growth factors receptors, such as the receptor for insulin-like growth factor-I, modulate the transcriptional activity of unliganded ERs (Mendez and Garcia-Segura, 2006; Garcia-Segura et al., 2010). However, it is unknown whether ER antagonists interfere with the regulation of ER activity by growth factors. The most plausible explanation is that ER antagonists are blocking the effect of locally synthesized estrogens, since aromatase, the enzyme that convert testosterone in estradiol, is expressed in the hypothalamus, including magnocellular neurons (Foidart et al., 1994; Naftolin et al., 2001; El-Eman Dief et al., 2012). In addition, the rodent brain expresses all the molecules necessary to synthesize testosterone from cholesterol (Garcia-Segura, 2008). Further studies should determine whether aromatase inhibitors affect the number of NADPH-diaphorase positive neurons in the SON and the PVN.

5. Conclusions

In summary, our findings indicate that ERs exert a differential effect on the regulation of nNOS in the SON and the PVN under acute osmotic stress conditions. This suggests that estradiol may regulate hydric balance by regulating NO production in the SON and the PVN.

Declaration of interest

The authors declare that there is no conflict of interest that would prejudice its impartiality.

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Author contributions

DG, GCP, LMGS and PC designed and supervised the experiments; DG, NL, MA and HP performed the experiments; DG prepared the figures for publication; DG and LMGS wrote the first draft of the manuscript; all authors read and approved the manuscript.

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Figure legends

Figure 1, Representative examples of histological sections showing NADPH-diaphorase positive cells in the supraoptic nucleus in a control animal injected with vehicle, and in animals injected with NaCl, estradiol and Nacl (E2 NaCl), ERα agonist and NaCl (PPT NaCl), ERα antagonist and NaCl (MPP NaCl), ERβ agonist and NaCl (DPN NaCl) or ERβ antagonist and NaCl (PHTPP NaCl). *, Optic chiasm. Scale bar 100 μm.

Figure 2, Representative examples of histological sections showing NADPH-diaphorase positive cells in the paraventricular nucleus in a control animal injected with vehicle, and in animals injected with NaCl, estradiol and Nacl (E2 NaCl), ERα agonist and NaCl (PPT NaCl), ERα antagonist and NaCl (MPP NaCl), ERβ agonist and NaCl (DPN NaCl) or ERβ antagonist and NaCl (PHTPP NaCl). *, Third ventricle. Scale bar 100 μm.

Figure 3, Number of NADPH-diaphorase positive cells in the supraoptic nucleus. Control, animals injected with vehicles; NaCl, animals injected with NaCl; E2 NaCl, animals injected with estradiol and NaCl; PPT NaCl, animals injected with the ERα agonist and NaCl; MPP NaCl, animals injected with the ERα antagonist and NaCl; DPN NaCl, animals injected with the ERβ agonist and NaCl; PHTPP NaCl, animals injected with the ERβ antagonist and NaCl. Data are represented as mean±SEM. ***,^,^^^ Statistical differences ***$p<0.001$ versus control values and ^$p<0.05$, ^^^$p<0.001$ versus NaCl values.

Figure 4, Number of diaphorase positive cells in the different subdivisions of the paraventricular nucleus: dorsal parvocellular (PaDC), lateral magnocellular (PaLM), parvocellular (PaV) and medial magnocellular (PaMM). Control, animals injected with vehicles; NaCl, animals injected with NaCl; E2 NaCl, animals injected with estradiol and NaCl; PPT NaCl, animals injected with the ERα agonist and NaCl; MPP NaCl, animals injected with the ERα antagonist and NaCl; DPN NaCl, animals injected with the ERβ agonist and NaCl; PHTPP NaCl, animals injected with the ERβ antagonist and NaCl. Data are represented as mean±SEM. **,***,^,^^^ Statistical differences **p<0.01;***p<0.001 versus control values, ^p<0.05; ^^^p<0.001 versus NaCl values.

Figure 1

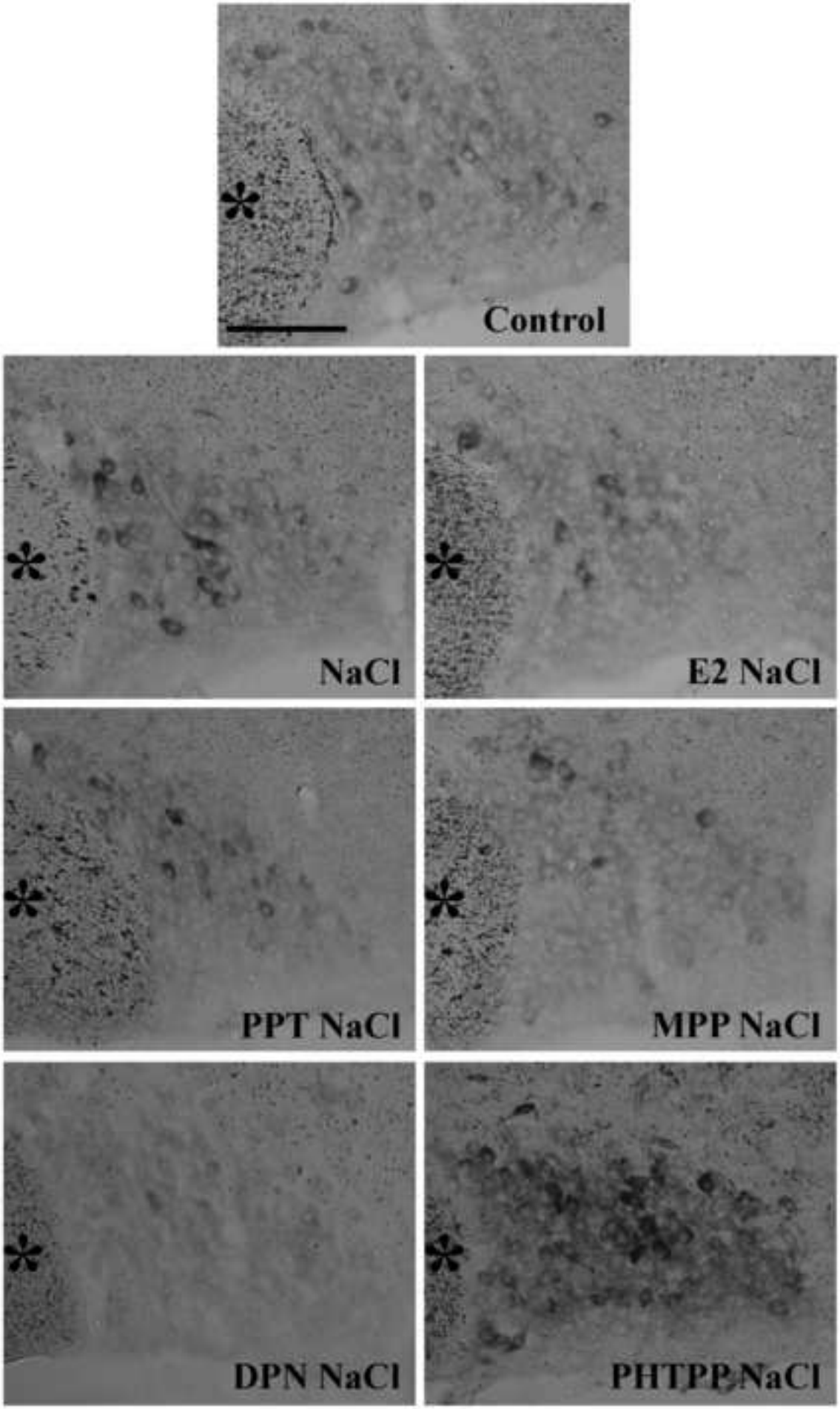

Figure 2

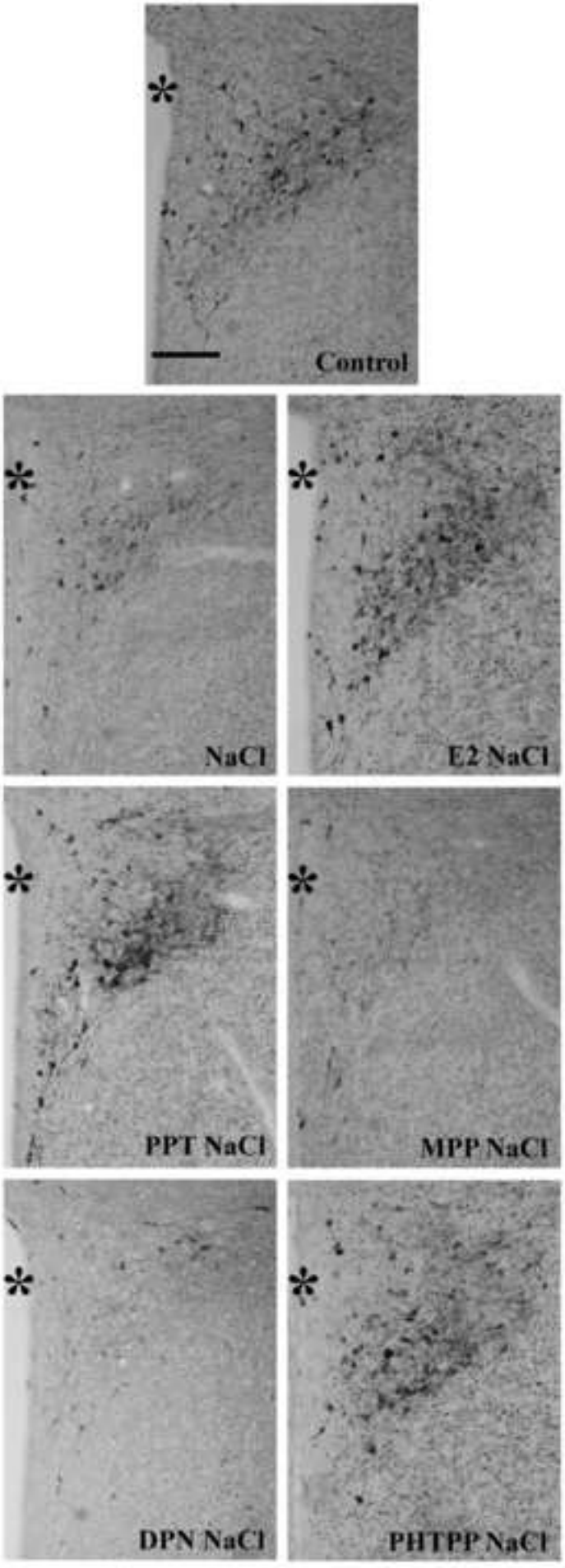

Figure 3

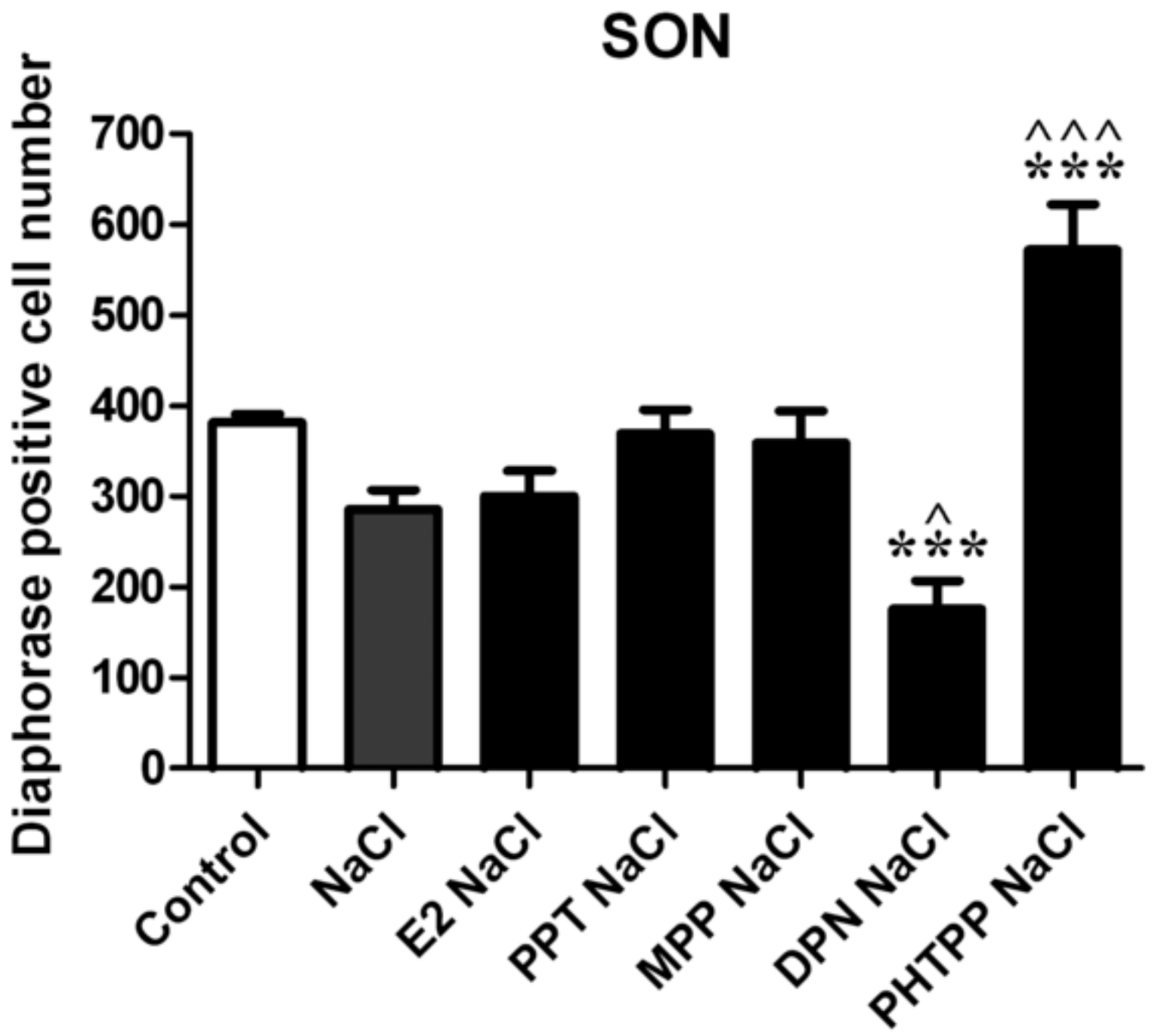

Figure 4

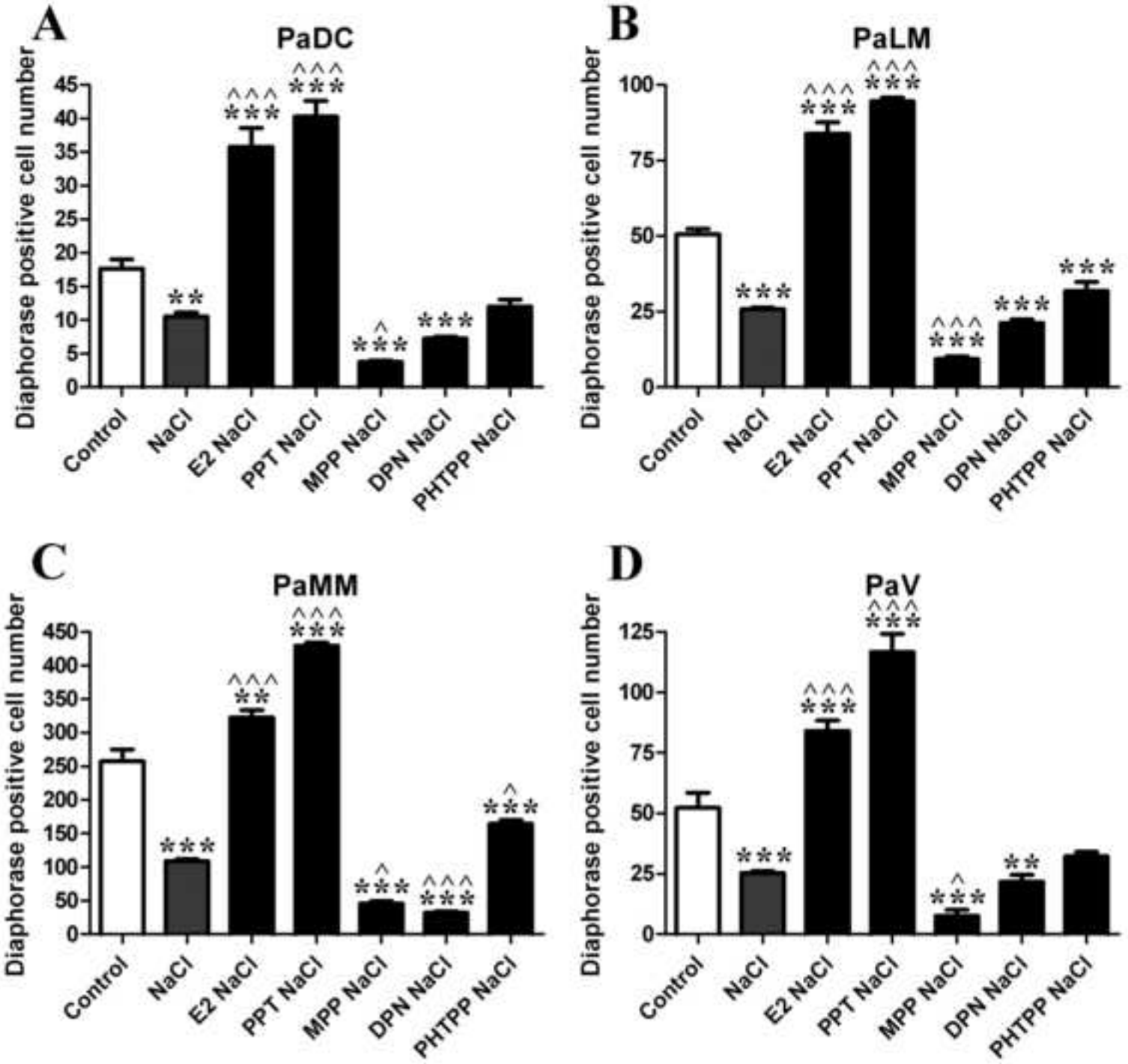

Table 1. Summary of the effect of the treatments on the number of NADPH-diaphorase positive cells in the supraoptic nucleus (SON), and in the four subdivisions of the paraventricular nucleus: dorsal parvocellular (PaDC); lateral magnocellular (PaLM); medial magnocellular (PaMM) and parvocellular (PaV). PPT, ERα agonist; MPP, ERα antagonist; DPN, ERβ agonist and PHTPP, ERβ antagonist. The green up-arrows indicate a significant increase and red down-arrows a significant decrease in the number of NADPH-diaphorase positive cells.

	SON	PaDC	PaLM	PaMM	PaV
NaCl		↓	↓	↓	↓
E_2 NaCl		↑	↑	↑	↑
ERα agonist-PPT NaCl		↑	↑	↑	↑
ERα antagonist-MPP NaCl		↓	↓	↓	↓
ERβ agonist-DPN NaCl	↓			↓	
ERβ antagonist-PHTPP NaCl	↑			↑	

Highlights

- Acute osmotic stress in OVX rats reduces the number of NADPH-d+ cells in the PVN
- Under these conditions estradiol increases the number of NADPH-d+ neurons
- Both ER and ER are involved in the regulation of the number of NADPH-d+ neurons