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L-Type calcium channels mediate water intake induced by angiotensin injection into median preoptic nucleus☆

Wilson Abrão Saad [a,b,c,e,*], Ismael Francisco Motta Siqueira Guarda [d], Luiz Antonio de Arruda. Camargo [c,e], Talmir Augusto Faria Brizola dos Santos [a], William Abrão Saad [f]

[a] Basic Institute of Biosciences – UNITAU, Taubaté, SP, Brazil
[b] Department of Exact and Natural Science, UNIARA Araraquara SP, Brazil
[c] Department of Physiology and Pathology, School of Dentistry, Paulista State University, UNESP Araraquara, SP, Brazil
[d] Department of Anesthesiology, Clinic Hospital State of São Paulo, São Paulo, Brazil
[e] Department of Physiology, Federal University of São Carlos SP, Brazil
[f] Department of Gastroenterology, Clinal Hospital of University of São Paulo USP, São Paulo SP, Brazil

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Abstract

Calcium ions are widely accepted as critically important in responses of neurons to a stimulus. We have show previously the central involvement of angiotensin II (ANGII) in water intake. This study determined whether voltage-dependent calcium channels are involved in ANGII-induced behavioral drinking implicating nitrergic mechanism. The antidipsogenic actions of L-type calcium channel antagonists nifedipine, on ANGII-induced drinking behavior were studied when it is injected into the median preoptic nucleus (MnPO). The influence of nitric oxide (NO) on nifedipine antidipsogenic action was also studied by utilizing the N^W-nitro-L-arginine methyl ester (L-NAME) a constitutive nitric oxide synthase inhibitor constitutive (cNOSI) and 7-nitroindazol (7-NIT) a specific neuronal nitric oxide synthase inhibitor (nNOSI) and L-arginine a NO donor. Rats 200–250 g, with cannulae implanted into MnPO, pre-treated into MnPO with either nifedipine, followed by ANGII, drank significantly less water than controls during the first 15 min after injection. However, L-NAME potentiated the dipsogenic effect of ANGII that is blocked by prior injection of nifedipine and L-arginine. 7-NIT injected prior to ANGII into MnPO also potentiated the dipsogenic effect of ANGII but with a less intensity than L-NAME that it is also blocked by prior injection of nifedipine. The results described in this paper provide evidence that calcium channels play important roles in the ANGII-induced behavioral water intake. The structures containing NO in the brain such as MnPO include both endothelial cells and neurons might be responsible for the influence of nifedipine on dipsogenic effect of ANGII. These data shows the correlation between L-type calcium channel and a free radical gas NO produced endogenously from amino acids L-arginine by endothelial and neuronal NO synthase in the control of ANGII-dipsogenic effect. This suggests that an L-type calcium channel participates in both short- and longer-term neuronal actions of ANGII by nitrergic way.

Keywords: Angiotensin II; Calcium channel; Nitric oxide drinking behavior; MnPO

1. Introduction

There is considerable evidence indicating an important role for the ANGII receptors of the areas of the central nervous system in the control of water and sodium intake (Antunes et al., 1998). Several data indicate that the MnPO is indeed the target of afferents from chemosensitive and barosensitive systems concerned with fluid homeostasis and cardiovascular regulation (O' neill and Brody, 1987; Yashuda et al., 2000).

☆ The Medical Ethics Committee of the Universidade Estadual Paulista UNESP approved all protocols in this study.

* Corresponding author. Department of Physiology and Pathology, School of Dentistry, Paulista State University, UNESP, 1680 Humaitá Street, Araraquara, SP 14801-903, Brazil. Tel.: +55 16 3301 6488; fax: +55 16 3322 4118.
E-mail address: saad@foar.unesp.br (W.A. Saad).

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Central injection of ANGII elicits prompt and pronounced responses such as increased thirst (Reid, 1988). The control of drinking behavior, endocrine and physiological responses induced by central ANGII have been demonstrated (Andersson et al., 1995; Wright and Harding, 1992). Intracerebroventricular (i.c.v.) injection of ANGII has been found to induce c-*fos* expression in a restricted number of sites in the forebrain and brainstem, such as a sharply defined lamina of neurons in the anterior region of the third ventricle (AV3V) including the MnPO, known to be concerned with the regulation of salt and water balance (Herbert et al., 1992; Xu and Herbert, 1994). Electrolytic lesions of this area inhibited the dipsogenic response to i.c.v. ANGII and also suppressed ANGII-induced c-*Fos* expression in supraoptic nucleus (SON), hypothalamic paraventricular nucleus (PVN), and MnPO suggesting that the MnPO might be a nodal site of action of ANGII in the brain (Xu and Herbert, 1995).

Nitric oxide (NO) participates of the central regulation of fluid and electrolytic balance. The electrolytic lesion of the lateral hypothalamic area (LH) reduces fluid, sodium intake, sodium urine excretion and the pressor effect induced by L-NAME, a NO synthase inhibitor, injected into MnPO. The involvement of both LH and MnPO in the actions of NO excitatory and inhibitory mechanisms related to water and sodium intake, sodium excretion and cardiovascular control is suggested (Saad et al., 2004).

It has been widely accepted that calcium ions are critically important for fast synaptic transmission, including the processes of neurotransmitter release from pre-synaptic terminals and post-synaptic receptor-mediated events (Ghosh and Greenberg, 1995). A major factor determining a neuronal Ca^{2+}-dependent signal is the opening of permeability pathways for Ca^{2+} in the cell membrane (Tsien et al., 1988).

The antidipsogenic actions of two L-type calcium channel blockers, verapamil and diltiazem has been demonstrated (Zhu and Herbert, 1997a). The interaction between nifedipine/NO on the MnPO hydromineral and cardiovascular regulation has not yet been demonstrated. Since NMDA-type glutamate receptors seem to be concerned in the rapid behavioral actions of ANGII (Zhu and Herbert, 1997a), the objectives of this work were to determine whether voltage-sensitive calcium channels are involved in ANGII-induced drinking behavior and to determine the possible participation of nitric oxide in the inhibitory effect of nifedipine on ANGII-induced drinking. Experiment 1 tested the action of nifedipine, L-type calcium channel antagonist, and L-arginine, NO donor, injected into MnPO on ANGII-induced water intake. Experiment 2 studied the influence of two nitric oxide synthase inhibitors: L-NAME (cNOSI) and 7-NIT (nNOSI) injected into MnPO on the inhibitory effect of nifedipine on ANGII-induced drinking.

2. Methods

2.1. Animals

Holtzman rats (from our laboratory) were housed in individual metabolic cages. Food (Purina Rat Chow) and tap water was available ad “libitum”. The temperature was maintained at 22±2 °C. The light cycle was held at 12:12 with lights on 06:00 h. All experiments were conducted during the light period, between 09:00 AM and 03:00 PM.

2.2. Drugs

1. Saline 0.15 M NaCl (control).
2. Angiotensin II (25 pmol/0.2 μl) (Sigma).
3. Nifedipine (17.22 nmol/0.2 μl or 34.22 nmol/0.2 μl) (Sigma).
4. L-NAME (10.80 nmol/0.2 μl) (Sigma).
5. 7-Nitroindazol (7-NIT) (6.52 nmol/0.2 μl) (Sigma).
6. L-Arginine (2.90 nmol/0.2 μl) (Sigma).

2.3. Cerebral surgery

Male Holtzman rats weighing 250–300 g were anesthetized with zoletil 50 mg/kg (tiletamine chloridrate 125 mg and zolazepan chlorhydrate 125 mg) into quadriceps muscle. A stainless steel cannula with 10- or 12-mm-long and 0.7-mm OD was implanted into the MnPO according to the coordinates of Paxinos and Watson (1986) atlas rat brain. The cannulae were fixed to the skull with the aid of jeweler screws and dental acrylic resin and protected with a stylet. After a 5-day recovery from surgery, the experiments started.

2.4. Drug injection

The drugs were injected into the MnPO by using a Hamilton micro syringe (5 μl) connected by a PE-10 polyethylene tubing (25 cm) to a needle (OD=0.3 mm), 0.5 mm longer than guide cannula, which was introduced into the brain through the cannula previously fixed to the animals’ head. The volume of injection was always 0.2 μl, injected over a period of 30–60 s.

2.5. Experimental protocol

The study of water intake was started 5 days after the brain surgery. The data were obtained from several batches of rats after the injection of the drugs into the MnPO of satiated animals.

2.5.1. Experiment 1

There were six groups of rats. Rats were given a MnPO injections of corresponding vehicle (saline 0.15 M NaCl; Group 1, $n=12$). Saline+ANGII 25 pmol (Group 2, $n=12$). Saline+Nifedipine 17.22 nmol (Group 3, $n=10$). Nifedipine 17.22 nmol followed 10 min later by 25 pmol ANGII (Group 4, $n=8$). Nifedipine 34.22 nmol followed 10 min later by 25 pmol ANGII (Group 5, $n=8$) and L-arginine 2.90 nmol followed 10 min later by 25 pmol ANGII (Group 6, $n=7$). After the second injection, water intake was measured every 15 min for 60 min using a graduated burette.

2.5.2. Experiment 2

There were seven groups of rats. Rats were given a MnPO injections of corresponding vehicle (saline 0.15 M NaCl; Group

1, $n=10$). Saline+ANGII 25 pmol (Group 2, $n=9$). Nifedipine 34.22 nmol followed 10 min later by 25 pmol ANGII (Group 3, $n=9$). L-NAME 10.80 nmol followed 10 min later by 25 pmol ANGII (Group 4, $n=8$). 7-NIT 6.52 nmol followed 10 min later by 25 pmol ANGII (Group 5, $n=8$). L-NAME 10.80 nmol+ Nifedipine 34.22 nmol followed 10 min later by 25 pmol ANGII (Group 6, $n=7$). 7-NIT 6.52 nmol+Nifedipine 34.22 nmol followed 10 min later by 25 pmol ANGII (Group 7, $n=7$). After the second injection, water intake was measured every 15 min for 60 min using a graduated burette.

2.6. Statistical analysis

The results are reported as mean±S.E.M for 1 h of water intake. The ANOVA and Dunnett's *t*-test were used to determine the significance between groups. The values were considered statistically significant at the 5% level ($P<0.05$).

2.7. Histology

At the end of the experiments, the rats were anesthetized with ether and perfused with saline and buffered formalin. The brains were removed, fixed in 10% formalin, frozen to −25 °C and cut into 20–30 μm coronal sections. Subjects were included in the analysis only if their cannula was in the lateral medial portion of MnPO (Fig. 1). We examined every section throughout the cannula path and eliminated all animals with cannula that perforated the ependyma, detected by histology and Evans blue injected before sacrifice.

3. Results

3.1. Experiment 1: the effects of nifedipine and L-arginine on water intake induced by injection of ANGII into MnPO (Fig. 2)

Rats of Group 1 (MnPO vehicle) consumed 0.4±0.1 ml h^{-1} of water. Rats of Group 2 (MnPO saline+ANGII) increased the water intake in the same period. Rats of Group 3 (MnPO saline+nifedipine) consumed the same amount of water compared with the control group. Rats of Group 4 (MnPO nifedipine 17.22 nmol+ANGII) decreased water intake compared with the SAL+ANGII group. Rats of Group 5 (MnPO nifedipine 34.22 nmol+ANGII) decreased the water intake compared with SAL+ANGII group with a higher intensity than the group 4. Rats of Group 6 (MnPO L-arginine+ ANGII) also decreased water intake. L-Arginine injected alone into MnPO produced no change in water intake. Rats consumed less than 1 ml of water in the second, third and fourth 15-min period regardless of treatments, and there was no difference between treatments. After the second injection, water intake was measured every 15 min for 60 min (Fig. 2).

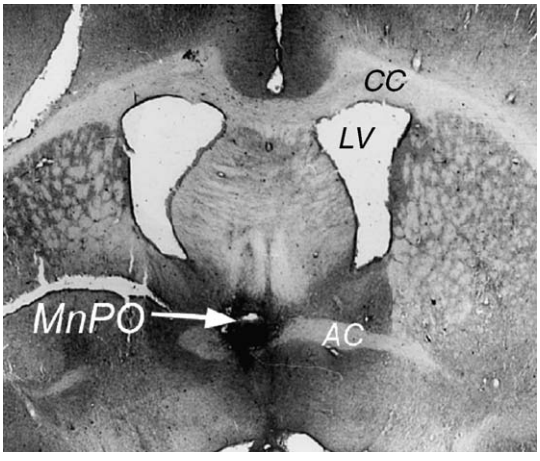

Fig. 1. Photomicrograph of a hematoxylin-stained transverse section of the rat brain showing site of injection into the MnPO (arrow). MnPO=median preoptic nucleus, CC=corpus callossum, LV=lateral ventricle, AC=anterior commissure.

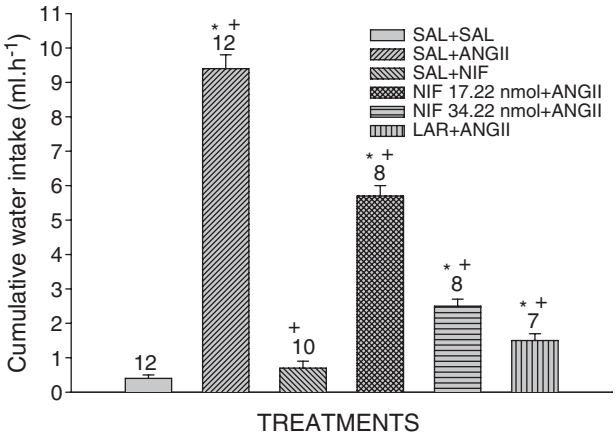

Fig. 2. Water intake during 60 min following MnPO injections of saline 0.15 M NaCl (SAL), SAL+ANGII, SAL+NIF, nifedipine 17.22 nmol or 34.22 nmol (NIF) followed by 25 pmol ANGII and l-arginine (LAR)+ANGII followed by 25 pmol ANGII. The number of animals at the top of each column. Data are means±S.E.M. $^{*}P<0.05$ vs. SAL+SAL, $^{+}P<0.05$ all group vs. NIF 34.22 nmol+ANGII (Dunnett's *t*-test).

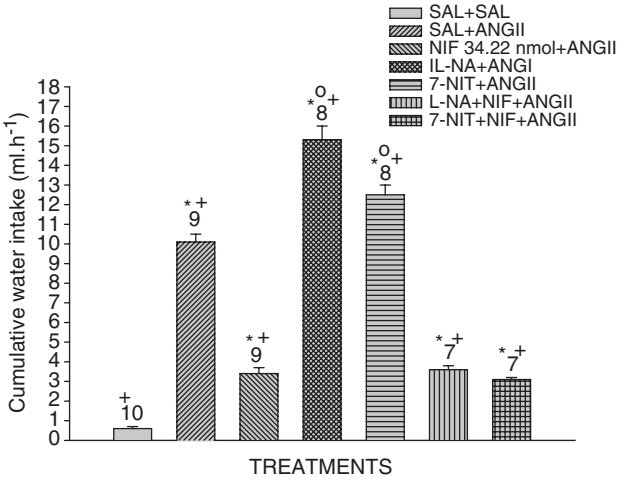

Fig. 3. Water intake during 60 min following MnPO injection of saline 0.15 M NaCl (SAL), SAL+ANGII, nifedipine 34.22 nmol (NIF) followed by 25 pmol ANGII, l-NAME (LNA)+ANGII, 7-nitroindazol (7-NIT)+ANGII, LNA+NIF+ ANGII and 7-NIT+NIF+ANGII. The number of animals at the top of each column. Data are means±S.E.M. $^{*}P<0.05$ vs. SAL+SAL, $^{+}P<0.05$ vs. NIF 34.22 nmol+ANGII and $^{O}P<0.05$ vs. SAL+ANGII (Dunnett's *t*-test).

3.2. Experiment 2: actions of L-NAME and 7-NIT injected into MnPO on nifedipine–ANGII antidipsogenic effects (Fig. 3)

Rats receiving injections of saline consumed 0.5± 0.01 ml h^{-1} of water intake. ANGII injected into MnPO increased water intake. Nifedipine injected prior to ANGII decreased water intake. L-NAME injected prior to ANGII potentiated the dipsogenic effect of ANGII. 7-NIT injected prior to ANGII also potentiated the dipsogenic effect of ANGII. Rats injected with L-NAME prior to nifedipine 34.22 nmol followed by ANGII consumed less water when compared with the values of L-NAME+ANGII. Rats injected with 7-NIT prior to nifedipine 34.22 nmol followed by ANGII also consumed less water intake when compared with the values of 7-NIT+ANGII. Rats consumed less than 1 ml of water in the second, third and fourth 15-min period regardless of treatments, and there was no difference between treatments. After the third injection, water intake was measured every 15 min for 60 min (Fig. 3).

4. Discussion

The L-type calcium channel complex contains at least four distinct binding sites (Catterall and Striessnig, 1992; Spedding and Paoletti, 1992). These are the dihydropyridine site, at which drugs such as nitrendipine, nifedipine, nimodipine, and isradipine (PN200-110) bind and compete with each other for binding; the phenylalkylamine site, at which verapamil and flunarizine bind; and the benzothiazepine site at which diltiazem binds, and a site to which 1,3-diphosphonates can bind (Rossier et al., 1989).

The results presented here indicated that ANGII-induced drinking behavior response may involve calcium channel regulation. Pretreated rats with nifedipine, which binds to the dihydropyridine site, reduced the dipsogenic response following MnPO injection of ANGII. The antidipsogenic effects of nifedipine were apparent in the first 15-min period following injection and were dose-dependent. Our study contrasts with the finding that tested the antidipsogenic actions of other two L-type calcium channel blockers, verapamil and diltiazem (Zhu and Herbert, 1997a), which bind to the phenylalkylamine and the benzothiazepine sites, respectively, and did not significantly suppress ANGII-induced drinking behavior as occurred with nifedipine. This suggest that ANGII my acts through a dihydropyridine-type calcium channel setting up an action potential which results in Ca^{2+} influx at the terminal, which controls release of neurotransmitters as has been demonstrated by Cohen and Weisbrod (1998).

The role of NO in the dipsogenic effect of ANGII also can involve vasopressin and L-type calcium channel as it has been demonstrated that NO has a modulator function of the hypothalamo–neurohypophyseal system (Kadekaro, 2004). NO-containing neurons involving several physiological functions have been localized in various parts of the central nervous system including MnPO and septal area (Zhu and Herbert, 1997b; Yang and Voogt, 2002; Saad et al., 2004). L-NAME, an endothelial and neuronal nitric oxide synthase inhibitor, potentiated the increase in water intake induced by ANGII injected into MnPO, whereas 7-NIT, a specific neuronal nitric oxide synthase inhibitor, also potentiated the dipsogenic effect of ANGII but with a significantly less intensity than L-NAME. This result indicates that endothelial and neuronal NO of the MnPO may be involved in the ANGII-induced drinking.

Nifedipine suppress ANGII-induced c-*Fos* expression more in the MnPO than in the OVLT, SFO and PVN (Zhu and Herbert, 1997a). The experiments utilizing nifedipine confirmed that L-type calcium channel is implicated in ANGII-dipsogenic effect by via nitrergic system; this can be confirmed by the results of this study that demonstrated that L-arginine, a NO donor, abolished the dipsogenic effect of ANGII with a significantly more intensity than nifedipine. We also propose that endogenous NO functions tonically as a modulator of brain neurotransmission. Previous studies have demonstrated that an NMDA receptor, one type of receptor-operated calcium channel, is implicated in ANGII-induced drinking behavior and c-*fos* expression (Xu et al., 1997). In most neurons of the central nervous system, there are at least two major classes of calcium channel: voltage-sensitive (VSCCs) and receptor-operated calcium channels (ROC). Nifedipine may have interfered with Ca^{2+} influx in the presynaptic terminals, where L-type calcium channels play important roles in the modulating presynaptic neurotransmitter release as has been demonstrate by Ghosh and Greenberg (1995). It may also have altered Ca^{2+}-dependent signaling events in postsynaptic neurons since previous studies have demonstrated the permissive effects of calcium channel voltage-sensitive (VSCCs) on NMDA receptor-mediated Ca^{2+} influx (Bumashev, 1996).

The results described in this paper provide evidence that calcium signaling plays important roles in ANGII-induced drinking response by implicating nitrergic pathways and L-type calcium channel. The effects of nifedipine on ANGII-induced c-*fos* expression suggest that calcium signaling may also participate long-term neuronal effects of ANGII, such as neuronal plasticity or the expression of 'late' genes as has been demonstrated by Zhu and Herbert (1997a). Thus, it may be that ANGII-induced drinking responses require activation of the dihydropyridine site on the L-type calcium channel complex, and that the presence of NO tonically modulates this behavior.

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References

Andersson B, Eriksson S, Rundgren M. Minireview: angiotensin and the brain. Acta Physiol Scand 1995;155:117–25.

Antunes VR, Camargo GMPA, Saad RG, Saad WA, Luiz AC, Camargo LAA. Role of angiotensin II and vasopressin receptors within the supraoptic nucleus in water and sodium intake induced by the injection of angiotensin II into the medial septal area. Braz J Med Biol Res 1998;31:1597–600.

Bumashev N. Calcium permeability of glutamate-gated channels in the central nervous system. Curr Opin Neurobiol 1996;6:311–7.

Catterall WA, Striessnig J. Receptor sites for Ca^{2+} channel antagonists. Trends Pharmacol Sci 1992;13:256–62.

Cohen RA, Weisbrod RM. Endothelium inhibits norepinephrine release from adrenergic nerves of rabbit carotid artery. Am J Physiol 1998;245:H871–8.

Ghosh A, Greenberg ME. Calcium signalling in neurons: molecular mechanisms and cellular consequences. Science 1995;268:239–47.

Herbert J, Forsling ML, Howes SR, Stacey PM, Shiers HM. Regional expression of c-*fos* antigen in the basal forebrain following intraventricular infusions of angiotensin and its modulation by drinking either water or saline. Neuroscience 1992;51:867–82.

Kadekaro M. Nitric oxide modulation of the hypothalamo–neurohypophyseal system. Braz J Med Biol Res 2004;37:441–50.

O' neill TP, Brody MJ. Role of the median preoptic nucleus in centrally evoked pressor responses. Am J Physiol 1987;252:R1165–72.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.

Reid JA. Actions of angiotensin II on the brain: mechanisms and physiological role. Am J Physiol 1988;246:F533–43.

Rossier JR, Cox JA, Niessor EJ, Bertzen CL. A new class of calcium entry blocker defined by 1,3-diphosphonates. J Biol Chem 1989;264:16598–607.

Saad WA, Gutierrez LI, Guarda IFSM, Camargo LAA, Santos TAF, Saad WA, et al. Lateral hypothalamus lesions influences water and salt intake, and sodium and urine excretion, arterial blood pressure induced by L-NAME and FK 409 injections into median preoptic nucleus in conscious rats. Life Sci 2004;75:685–97.

Spedding M, Paoletti R. Classification of calcium channels and the sites of action of drugs modifying channel function. Pharmacol Rev 1992; 44:363–76.

Tsien RW, Lipscombe D, Madison DV, Bley KR, Fox AP. Multiple types of neuronal calcium channels and their selective modulation. Trends Neurosci 1988;11:431–8.

Wright JW, Harding JW. Regulatory role of brain angiotensins in the control of physiological and behavioral responses. Brain Res Rev 1992;17:227–62.

Xu Z, Herbert T. Regional suppression by water intake of c-*fos* expression induced by intraventricular infusions of angiotensin II. Brain Res 1994; 659:157–68.

Xu Z, Herbert T. Regional suppression by lesions in the anterior 3rd ventricle of c-*fos* expression induced by either angiotensin-II and hypertonic saline. Neuroscience 1995;67:35–47.

Xu Z, Mal1on JM, Zhu B, Herbert I. NMDA receptors may mediate dipsogenic responses and c-*fos* expression induced by intracerebroventricular infusion of angiotensin II. Neuroscience 1997;78:203–14.

Yang SP, Voogt JL. Mating-activated nitric oxide-producing neurons in specific brain regions in the female rat. Brain Res 2002;20:79–87.

Yashuda Y, Honda K, Negoro H, Higuchi T, Goto Y, Fukuda S. The contribution of the median preoptic nucleus to renal sympathetic nerve activity increased by intracerebroventricular injection of hypertonic saline in rat. Brain Res 2000;867:107–14.

Zhu B, Herbert J. Calcium channels mediate angiotensin II-induced drinking behaviour and c-*fos* expression in the brain. Brain Res 1997a;778:206–14.

Zhu B, Herbert J. Angiotensin II interacts with nitric oxide cyclic GMP pathway in the central control of drinking behavior: mapping with c-*fos* and NADPH-diaphorase. Neuroscience 1997b;79:543–53.