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DIFFERENTIAL CARDIOVASCULAR AND RENAL RESPONSES PRODUCED BY MICROINJECTION OF THE KAPPA OPIOID, U-50488H, INTO SUBREGIONS OF THE PARAVENTRICULAR NUCLEUS.

Helmut B. Gottlieb[1], Kurt J. Varner [1], Velga A. Kenigs[1], Antonio M. Cabral[2] and Daniel R. Kapusta[1]

[1] Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, LA, USA, 70112

[2] Department of Physiological Sciences, Federal University of Espirito Santo, Vitoria, Brazil, 29040-090.

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Corresponding Author and Address for Reprint Requests:

Daniel R. Kapusta, Ph.D.

Department of Pharmacology and Experimental Therapeutics

Louisiana State University Health Sciences Center

1901 Perdido St.

New Orleans, LA 70112

Phone (504) 568-4740

FAX (504) 568-2361

E-mail: dkapus@lsuhsc.edu

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ABSTRACT

Kappa opioids produce a centrally mediated diuresis, antinatriuresis and renal sympathoexcitation *in vivo*, however the specific brain sites mediating these responses are unknown. This study examined the role of the hypothalamic paraventricular nucleus (PVN) and the renal sympathetic nerves in mediating the cardiovascular and renal responses to central kappa opioid receptor activation. In ketamine/xylazine-anesthetized rats, bilateral microinjection of the selective kappa agonist, U-50488H (100 ng) into the posterior magnocellular division of the PVN significantly increased urine flow rate (control, 47 ± 9 μl/min; 40-min, 108 ± 10 μl/min) without changing urinary sodium excretion or cardiovascular function. In other animals, microinjection of U-50488H into the same site elicited a similar water diuresis without a change in renal sympathetic nerve activity. In contrast, microinjection of U-50488H (100 ng) into the parvocellular PVN produced an immediate pressor response (Δ 16±3 mmHg) that occurred with a potential baroreflex evoked bradycardia (Δ -26±8 bpm), renal sympathoinhibition (Δ -18±4 %), natriuresis (Δ 38±1 %), and delayed (30-min) antidiuresis (Δ -22±9 %). These results were prevented by pretreatment with the kappa receptor antagonist, nor-binaltorphimine, and not obtained when U-50488H was injected outside the PVN, or when vehicle was injected into the PVN. Together, these results demonstrate that the posterior magnocellular PVN is a brain site where central kappa opioids act to produce diuresis, presumably by inhibiting the secretion of arginine vasopressin. Alternatively, central kappa opioids evoke antinatriuresis via augmenting renal sympathetic nerve activity and/or other neurohumoral sodium retaining pathways at brain sites other than the hypothalamic PVN.

The administration of kappa opioid agonists (e.g., spiradoline, enadoline, dynorphin A(1-17), U-50488H [(trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzene-acetamide) methane sulfonate], etc.) produces a marked diuretic response in different animal species and man (Leander et. al., 1985; 1987; Ashton et al., 1989; Rimoy et al., 1991; Kapusta and Obih, 1993). The kappa opioid-evoked increase in urine flow rate is unique in that it is not associated with a concurrent increase in urinary sodium or potassium excretion (Leander et. al., 1985; 1987; Salas et al., 1989; Rimoy et al., 1991; Kapusta and Obih, 1993); i.e., kappa opioids produce a selective water diuresis (for review see Kapusta, 1995). Findings in rats indicate that kappa opioids affect the renal excretion of water and electrolytes, at least in part, by an action in the periphery (Salas et al., 1989; 1992; Slizgi and Ludens, 1986; Blackburn et al., 1986; Wang et al., 1994). However, considerable evidence in this and other species have shown that kappa opioids produce diuresis primarily by a central action to inhibit the secretion of vasopressin into the systemic circulation (Carter and Lightman, 1984; Leander et al., 1985; Oiso et al., 1988; Yamada et al., 1989; Brooks et al., 1991a,b; 1993). Despite evidence supporting a central action, the specific brain site(s) where kappa opioids act to affect the renal handling of water and electrolytes has not been established *in vivo*.

Using compartmentalized rat hypothalamoneurohypophysial explants in culture, Rossi and Brooks (1996) showed that the activation of hypothalamic kappa receptors prevented the osmotically stimulated release of vasopressin. However, in these studies the precise location of the hypothalamic kappa opioid receptors involved was not determined. Also unknown is whether these same systems (e.g., involving vasopressin) are involved in producing the diuretic responses elicited by kappa opioids *in vivo*. In fact, the hypothalamic explant used in the culture system did not contain the paraventricular hypothalamic (PVN) nucleus. This is of merit since the

magnocellular region of the PVN contains a large number of neurons that synthesize and release vasopressin in the pituitary (Watson et al., 1982; Poulain and Wakerly, 1982; Mansour et al., 1996). In addition, there is a high concentration of the native kappa ligand, dynorphin A(1-17), localized in the hypothalamus (Holt et al., 1980; Watson et al., 1982) and kappa opioid receptors are densely expressed in the magnocellular and parvocellular regions of the PVN (Mansour et al., 1996). In addition to its well known role with vasopressin, the parvocellular region of the PVN also participates in the regulation of central sympathetic outflow (Swanson and Kuypers, 1980; Strack et al. 1989). This latter point is of interest since concurrent with an increase in urine flow rate, central kappa opioids also increase renal sympathetic nerve activity and produce a renal nerve-dependent decrease in urinary sodium excretion (Kapusta and Obih, 1993; 1995). Thus, the PVN is a brain site in which kappa opioids might act *in vivo* to produce diuresis, antinatriuresis and renal sympathoexcitation.

To study the CNS control of renal function in rats during experimental procedures that require anesthesia (e.g., glass multibarrel pipette stereotaxic microinjection procedures), our laboratory developed a protocol that uses the i.v. infusion of the α_2-agonist xylazine to enhance basal levels of urine flow rate and urinary sodium excretion in ketamine-anesthetized rats (Cabral et al., 1997; 1998; Menegaz et al., 2000; 2001). Using this approach, we have shown that the i.c.v. administration of kappa opioids produces diuretic and antinatriuretic responses in ketamine/xylazine-anesthetized rats which are similar in magnitude and time course to those observed in conscious animals (Cabral et al., 1997). In addition, drug-evoked increases in multifiber renal sympathetic nerve activity can be readily measured in this model (Menegaz et al., 2001).

Therefore, the purpose of this study was to examine the changes in cardiovascular and renal excretory function produced by the bilateral microinjection of the selective kappa opioid receptor agonist, U-50488H, into the PVN of ketamine/xylazine-anesthetized rats. Since kappa opioid receptors are expressed in both magnocellular and parvocellular regions of the PVN, these studies also compared the cardiovascular and renal responses produced by bilateral microinjection of U-50488H into these anatomically and physiologically distinct regions. Finally, in certain studies, changes in renal sympathetic nerve activity were also measured to determine whether activation of kappa opioid receptor pathways in the magnocellular/parvocellular PVN may affect cardiovascular and/or renal excretory function via altering central sympathetic outflow to the kidneys.

METHODS

Subjects

Experiments were performed using male Sprague-Dawley rats (270-350 g, Harlan Inc., Indianapolis, IN). The rats were group housed in a temperature and humidity-controlled room with a 12-hour light/dark cycle. Standard rat chow (sodium content 163 meq/kg) and tap water were available *ad libitum.* All experimental procedures were conducted in accordance with the Louisiana State University Health Sciences Center and the national Institutes of Health guidelines for the care and use of animals.

General Surgical Procedures

On the day of the experiment, rats were anesthetized with sodium methohexital (Brevital; 35 mg/kg, i.p., supplemented with 10 mg/kg, i.v. as needed; King Pharmaceuticals, Bristol, TN)

and implanted with chronic indwelling catheters (PE-10 connected to PE-50, Becton Dickinson and Company, Sparks, MD) into the femoral artery and vein for measurement of arterial blood pressure and infusion of isotonic saline vehicle/drugs, respectively. Through a suprapubic incision, a catheter (flanged PE-240, Becton Dickinson and Company, Sparks, MD) was also implanted into the urinary bladder for collection of urine samples. All catheters were exteriorized and securely sutured to adjacent muscle and skin. The arterial and venous catheters were connected to a pressure transducer (model P23Db, Statham, Oxnard, CA) and an infusion pump (model 944, Harvard apparatus, South Natick, MA), respectively. The mean and pulsatile arterial pressures were recorded on a grass model 7 polygraph (Grass instruments, Quincy, MA). Heart rate was determined from the arterial pressure signal by a Grass model 7P4 Tachograph.

After implantation of these catheters, certain rats (still anesthetized with sodium methohexital) were also implanted with a recording electrode on a renal nerve bundle for direct measurement of multifiber renal sympathetic nerve activity using techniques described previously (Kapusta and Obih, 1993;1995; Menegaz et al., 2001). The left kidney was exposed through a left incision via a retroperitoneal approach. With the use of a dissecting microscope (25 X), a renal nerve branch from the aorticorenal ganglion was isolated and carefully dissected. The renal nerve branch was then placed on a bipolar platinum wire (Cooner Wire Company, Chatsworth, CA) electrode. Renal sympathetic nerve activity was amplified (10,000-50,000 X) and filtered (low, 30; high, 3000 Hz) with a Grass P511 Bandpass Amplifier (Quincy, MA). When an optimal renal sympathetic nerve activity signal was observed, the recording electrode was fixed to the renal nerve branch with a dental impression material (Coltene President). The electrode cable was then secured in position by suturing it to the abdominal trunk muscles. Finally, the electrode cable was exteriorized, and the flank incision was closed in layers. The

amplified and filtered signal was channeled to a Tektronix 5113 Oscilloscope (Tektronix, Beaverton, OR) and Grass model 7DA polygraph for visual evaluation, to an audio amplifier-loudspeaker (Grass model AM 8 Audio Monitor) for auditory evaluation, and to a rectifying voltage integrator for quantification (Grass model 7P10). The integrated voltage signals were displayed on the Grass polygraph and data acquisition for renal sympathetic nerve activity measurements were performed with a commercially available software package (Acknowledge version 3.7.1 for Windows, Biopac Inc., Santa Barbara, CA). Integrated renal sympathetic nerve activity was expressed as microvolt-seconds per 1-sec intervals. For each 10-min control and experimental period, the values for integrated renal sympathetic nerve activity were sampled over the entire collection period and the numbers were averaged. The data for renal sympathetic nerve activity are expressed as the percent of the baseline value obtained during the control period during ketamine/xylazine infusion with this being expressed as 100% for each animal. We determined the background noise level of renal sympathetic nerve activity by observing the neural signal that remained 10 min after the start of the intravenous infusion of ketamine and xylazine; this value was then subtracted from all control and experimental values of renal sympathetic nerve activity.

After implantation of catheters and in certain animals a renal nerve recording electrode under sodium methohexital anesthesia, rats were administered a loading dose of ketamine (40 mg/kg, i.v.) over a 5-min period. An i.v. infusion (55 μl/min) of isotonic saline containing ketamine (1.0 mg/kg/min) and xylazine (80 μg/kg/min) was then started and continued throughout the experiment as previously described (Cabral et al., 1997; 1998; Menegaz et al., 2000; 2001). Ketamine and xylazine-anesthetized rats were then placed prone in a stereotaxic apparatus with the bite bar 3.5 mm below the interaural line. The skull was then partially

removed between the bregma and lambda, and the dura was carefully dissected parallel to the sinus vein. Drugs were microinjected bilaterally into the PVN using a three-barrel glass micropipette (0.4 mm I.D., 0.75 mm O.D.) with a composite tip diameter of less than 40 μm. The coordinates for microinjection of drugs/vehicle into the right and left PVN (2.0 mm posterior to bregma, 0.4 mm lateral to either side of the midline, and 7.9 mm below the surface of the skull) were obtained from the atlas of Paxinos and Watson (1998). The barrels of the pipettes were filled by vacuum with 1% solution of isotonic saline vehicle or the kappa opioid receptor antagonist, nor-binaltorphimine (barrel 1), the selective kappa opioid agonist, U-50488H (barrel 2) and pontamine sky blue dye (barrel 3). All drugs were injected in a volume of 60 nl over a period of 0.5-1 min by using a pneumatic pressure injection system (Pneumatic Picopump, World Precision Instruments, Inc, Sarasota, Fl, USA). The speed and volume of the injection was controlled by watching the movement of the fluid meniscus in the pipette with a stereomicroscope and a gradicule affixed to the pipette.

For certain studies, drugs were microinjected (glass multibarrel pipettes) into the right lateral cerebroventricle of ketamine and xylazine-anesthetized rats instead of the PVN. The coordinates for i.c.v. microinjection were 0.3 mm posterior to the bregma, 1.3 mm lateral to the midline and 5.5 mm below the skull surface (Paxinos and Watson, 1998). Verification of pipette position in the lateral cerebroventricle was made by observing injected dye in the lateral ventricle following completion of the study and subsequent postmortem brain section (Kapusta and Obih, 1993, 1995; Kapusta et al., 1997; Menegaz et al., 2001).

Experimental protocols

Studies were performed to determine the cardiovascular and renal excretory responses produced by bilateral microinjection of the selective kappa agonist, U-50488H (10 or 100 ng), into the magnocellular region of the PVN of ketamine/xylazine-anesthetized rats. Renal sympathetic nerve activity was not measured in these studies. The i.v. infusion of xylazine enhances the renal excretion of water and sodium, and these levels tend to stabilize and remain constant approximately 120 min from the beginning of infusion (Cabral et al., 1997; 1998; Menegaz et al., 2000, 2001). Therefore, after completion of surgery and stabilization of cardiovascular and renal excretory responses, two consecutive control urine samples were collected (10 min each). The selective kappa opioid agonist, U-50488H (10, n = 6; or 100 ng, n = 8), was then microinjected bilaterally into the PVN. The drug was allowed 5 minutes for distribution. The experimental period then entailed measurement of cardiovascular parameters and collection of urine for 80-min (consecutive 10-min periods).

Additional studies were performed in separate ketamine/xylazine-anesthetized rats to determine whether changes in renal sympathetic nerve activity are involved in producing the cardiovascular and/or renal excretory responses produced by the microinjection of U-50488H into the magnocellular or parvocellular regions of the PVN. For these studies a renal sympathetic nerve recording electrode was implanted in each rat. As described above, the experimental protocol entailed collection of consecutive 10-min urine samples before (control, 20-min) and after (experimental, 80-min) the bilateral microinjectin of U-50488H into the PVN. For these studies, only a single dose of U-50488H (100 ng/60 nl) was tested. For data analysis, rats whose microinjection sites were either located bilaterally in either the magnocellular (group 1; n= 5) or the parvocellular (group 2; n=8) region of the PVN were grouped respectively and considered to

be 'within' the PVN. Alternatively, animals were grouped together if the microinjection sites were located bilaterally 'outside' of the PVN. As an additional control, studies were performed in which isotonic saline vehicle (60 nl) was microinjected bilaterally into the magnocellular or parvocellular regions of the PVN of ketamine/xylazine-anesthetized rats.

Studies were also conducted to determine the receptor selectivity of the cardiovascular and renal responses produced by microinjection of U-50488H into the PVN. For these studies, following control urine collections, ketamine/xylazine-anesthetized rats were microinjected bilaterally with the kappa opioid receptor antagonist, nor-BNI (100 ng/60 nl = 2.26 mM), into the magnocellular or parvocellular PVN. Immediately following antagonist injection, urine was collected during a 10-min experimental nor-BNI pretreatment period. Next, following nor-BNI pretreatment (10-min), U-50488H (100 ng/60 nl = 4.1 mM) was microinjected bilaterally into the same region of the PVN. Following U-50488H injection, consecutive urine samples (10-min ea.) were immediately collected for 80-min.

At the end of all microinjection experiments, injection sites were marked bilaterally by microinjecting pontamine sky blue dye (60 nl) through the third barrel of the pipette. In the dose-response studies with U-50488H described above, only the rats whose microinjection sites were located bilaterally within the magnocellular region of the PVN or completely outside of the PVN were used for data analysis. Similarly, data analysis for the renal nerve recording studies only included animals in which it was demonstrated that U-50488H was injected bilaterally into sites within the magnocellular (group 1) or parvocellular (group 2) regions of the PVN.

For the investigations outlined above, U-50488H was selected for use since it is a highly selective agonist of the kappa opioid receptor, which has approximately 60 times more affinity for the kappa-1 receptor as compared to mu-1 receptors and 80 times more as compared to mu-2

and delta receptors (Clark et al., 1989). The kappa opioid receptor antagonist, nor-binaltorphimine (nor-BNI), was used in these studies to demonstrate the receptor selectivity of U-50488H on cardiovascular and renal function. In the present studies, the doses of U-50488H (10 and 100 ng/60 nl) and nor-BNI (100 ng/60 nl) for PVN microinjection studies was based on previous findings. In these studies, we showed that the diuretic, antinatriuretic and renal sympathoexcitatory responses produced by the i.c.v. administration of U-50488H (1 μg) or the endogenous kappa ligand, dynorphin A(1-17) (10 μg), were exclusively mediated by an action of the drug on kappa opioid receptors, since these renal excretory and renal nerve responses were completely blocked by the i.c.v. pretreatment of animals with nor-BNI (1 μg), a highly selective kappa opioid receptor antagonist (Kapusta and Obih, 1993; 1995; Kapusta et al., 1997). Thus, in the present studies, nor-BNI and U-50488H were microinjected into the magnocellular or parvocellular regions of the PVN at a concentration ration of 1:1, but at substantially lower doses than those used for i.c.v. injection studies.

Histological processing

At the end of the microinjection study, anesthetized rats were perfused transcardially with normal saline followed by fixative (4% paraformaldehyde in phosphate buffered saline, pH =7.4). The brain was then removed and post-fixed with 8% paraformaldehyde and 20% sucrose at 4° C for at least 3 days. The brain was frozen and cut in 60 μm coronal sections using a cryostat microtome (Leica, CM1900). All sections were collected in gel alcohol, mounted to glass slides and allowed to dry in a slide warmer. Then, the sections were stained with 1% neutral red, cover slipped, and allowed to dry. The microinjections sites were identified

microscopically from stained sections using the atlas of Paxinos and Watson (1998) as a reference.

Data Analysis

Changes in heart rate and mean arterial pressure produced by drug administration were calculated directly from the polygraph tracings and through a computer driven data acquisition software (Acknowledge 3.5, Biopac Systems, Santa Barbara, CA). Urine volume was determined gravimetrically and the urinary sodium concentration was measured by flame photometry (IL943 automatic flame photomoter, Instrumentation Laboratory).

All data are expressed as means ± S.E. Group data were statistically analyzed using a one-way repeated measures analysis of variance (ANOVA) for the main effects and interactions and a Dunnett's post hoc test for pairwise comparisons among the means. In each case, statistical significance was defined as $P < 0.05$.

Drugs

The drugs used in this study were nor-binaltorphimine (Nor-BNI; Research Biochemicals, Natick, MA), U-50488H (UpJohn Laboratories, Kalamazoo, MI), sodium methohexital (King Pharmaceuticals, Bristol, TN), ketamine hydrochloride (Phoenix Scientific, Inc., St. Joseph, MO), xylazine (Butler Corp., Columbus, OH), and phenylephrine hydrochloride (Sigma-Aldrich, Inc., St. Louis, MO).

RESULTS

Figure 1 illustrates the cardiovascular, renal excretory, and renal sympathetic nerve responses produced by i.c.v. administration of the selective kappa opioid agonist, U-50488H

(24.6 nmol = 10 μg), or isotonic saline vehicle in ketamine/xylazine-anesthetized Sprague-Dawley rats. The i.c.v. injection of isotonic saline vehicle did not alter any parameter over the course of the experiment. In contrast, as compared to respective control (C) levels (V, 76 ± 7 μl/min; UNaV, 8.2 ± 0.6 μeq/min) i.c.v. injection of U-50488H increased urine flow rate (40-min, 136 ± 9 μl/min) and decreased urinary sodium excretion (40-min, 3.5 ± 0.3 μeq/min). The diuretic and antinatriuretic responses were both significantly ($p<0.05$) different from respective control levels 20 min after drug injection with the diuretic, but not antinatriuretic response returning to baseline values by 70-min post drug injection. I.c.v. U-50488H also increased renal sympathetic nerve activity within minutes following drug injection, but the renal sympathoexcitatory response was not significantly elevated until 30-min following drug injection. The i.c.v. administration of U-50488H failed to produce a significant change in heart rate or mean arterial pressure over the 80-min experiment.

The cardiovascular and renal excretory responses produced by the bilateral microinjection of U-50488H (10 or 100 ng) into the magnocellular PVN of ketamine/xylazine-anesthetized rats are shown in Figure 2. The microinjection of 10 or 100 ng of U-50488H into the magnocellular PVN elicited a significant ($p<0.05$) increase in urine flow rate, with the diuresis elicited by the higher dose starting earlier (20-min) and persisting for a longer duration before gradually decreasing towards basal values. The lower dose caused a similar diuretic effect, but of a delayed onset and faster return to control levels. The diuretic response was maximal by 30 min for the 10 ng dose (C, 60 ± 6; 30-min, 95 ± 12) and 40 min for the 100 ng dose (C, 48 ± 12 μl/min; 40-min, 108 ± 13 μeq/min). In contrast to the changes in urine flow rate, the bilateral microinjection of U-50488H into the magnocellular PVN did not alter heart rate, mean arterial pressure or urinary sodium excretion at any time period. At the lower dose (10

ng), urinary sodium excretion tended to increase 20-min following drug microinjection, but this change was not statistically significant ($p>0.05$). Similarly, microinjection of U-50488H into brain regions entirely outside of the PVN failed to alter any cardiovascular or renal excretory parameter over the course of the study.

Additional experiments were performed in separate animals to further investigate the cardiovascular and renal responses produced by microinjection of U-50488H (100 ng) into the posterior magnocellular PVN and determine whether changes in any of these parameters were associated with alterations in renal sympathetic nerve activity. As shown in Fig. 3, bilateral microinjection of U-50488H into this brain region produced a selective increase in urine flow rate ($p<0.05$), which occurred without altering urinary sodium excretion. The pattern and magnitude of the changes in urine flow rate were similar to those observed in separate ketamine/xylazine-anesthetized rats (Fig. 2). Despite producing diuresis, the bilateral microinjection of U-50488H into the magnocellular PVN did not alter renal sympathetic nerve activity at any time point. In other animals, bilateral pretreatment of the magnocellular PVN with the selective kappa opioid receptor antagonist, nor-BNI (100 ng) completely blocked the diuretic response produced by the subsequent microinjection of U-50488H into the same brain site (Fig. 3). When administered alone, microinjection of nor-BNI (100 ng) into the magnocellular PVN failed to alter any cardiovascular or renal parameter (data not shown). In these investigations, the microinjection of U-50488H (100 ng) into regions outside of the PVN did not alter any experimental parameter (Fig. 3). Moreover, the bilateral microinjection of isotonic saline vehicle (60 nl) into the magnocellular region of the PVN of ketamine/xylazine-anesthetized rats did not alter any cardiovascular or renal parameters (data not shown).

Figure 4 shows the cardiovascular, renal excretory, and renal sympathetic nerve activity responses produced by the bilateral microinjection of U-50488H (100 ng) into the medial/lateral parvocellular regions of the PVN of naïve rats, or rats pretreated with the kappa opioid receptor antagonist, nor-BNI. For comparison, the responses produced by the microinjection of U-50488H into regions outside of the PVN are also illustrated (same data as shown in Fig. 3). The bilateral microinjection of U-50488H into the medial/lateral parvocellular division of the PVN evoked an immediate (10-min), but transient increase in mean arterial pressure which returned to control levels 30 min after drug injection. Concurrent with the pressor response, microinjection of U-50488H into the parvocellular PVN significantly ($p<0.05$) decreased heart rate and renal sympathetic nerve activity, and increased urinary sodium excretion. In addition, microinjection of U-50488H into the parvocellular PVN evoked a delayed antidiuresis that occurred 30-40 min post drug injection. As previously noted, microinjection of U-50488H into regions outside of the PVN (Figs. 3 and 4), or isotonic saline vehicle (60 nl) into the parvocellular PVN (data not shown) of ketamine/xylazine-anesthetized rats did not alter any cardiovascular or renal parameters. In other animals, bilateral pretreatment of the parvocellular PVN with the selective kappa opioid receptor antagonist, nor-BNI (100 ng) completely blocked the cardiovascular and renal response produced by the subsequent microinjection of U-50488H into the same brain site (Fig. 4). When administered alone, microinjection of nor-BNI (100 ng) into the parvocellular PVN failed to alter any cardiovascular or renal parameter (data not shown).

The histological identified sites into which U-50488H (100 ng) was microinjected into the PVN of animals depicted in Figures 3 (magnocellular) and 4 (parvocellular) are shown in Figure 5. In addition, the sites in which U-50488H was injected into regions outside of the PVN are also shown in Figure 5.

DISCUSSION

The present study showed that bilateral microinjection of the highly selective kappa opioid receptor agonist, U-50488H, into the magnocellular region of the hypothalamic PVN of anesthetized rats produced a selective increase in the renal excretion of water, but not sodium. While strong evidence suggests that kappa opioids may elicit a water diuresis from a locus within the hypothalamus (Rossi and Brooks, 1996; Rossi et al., 1997), the present findings establish that the magnocellular region of the PVN is an important brain site in which central kappa agonists act to elicit a solute free water diuresis *in vivo*.

In different species the diuretic effect of kappa opioids has been associated with an inhibition of circulating vasopressin levels (Carter and Lightman, 1984; Leander et al., 1985; 1987; Oiso et al., 1988; Yamada et al., 1989; Brooks et al., 1991a,b; 1993). Following systemic administration, kappa opioids could inhibit the release of vasopressin by stimulating kappa opioid receptors located on the posterior pituitary (Zho et al., 1988; Arvidsson et al., 1995, Mansour et al., 1996; Rossi and Brooks, 1996). However, since kappa opioids with low ability to permeate the brain show only negligible diuretic effects, it has been suggested that kappa opioids act primarily at sites within the blood-brain barrier to inhibit vasopressin secretion and produce diuresis (Brooks et al., 1993). A hypothalamic site is further suggested by studies performed by Rossi and Brooks (1996), which studied hypothalamoneurohypophysial explants in culture. In these studies, the kappa opioid agonist, BRL-52656 (S(-)-2-(1-pyrrolidinylmethyl)-1-(4-trifluoromethylphenyl) acetyl piperidine hydrochloride), inhibited osmotically stimulated vasopressin secretion in a selective kappa-opioid receptor dependent fashion (nor-BNI sensitive) only when applied to the hypothalamic compartment (Rossi and Brooks, 1996). In contrast, while BRL-52656 also diminished vasopressin secretion when added to the compartment

containing the neurohypophysis, a substantially larger concentration of the agonist was required and the drug response was shown to involve activation of classical opioid receptors other than the kappa subtype (naloxane sensitive) (Rossi and Brooks, 1996). Similarly, in related studies AVP mRNA content paralleled the changes in AVP secretory rate induced by hypothalamic kappa-receptor activation, but was unaltered when the kappa agonist, niravoline (RU 51599) was applied to the neural lobe (Rossi et al., 1997). Together, these findings provide strong evidence that kappa opioid ligands activate selective kappa opioid receptor pathways within the brain, most likely at the level of the hypothalamus, to inhibit vasopressin secretion and subsequently produce diuresis.

In the present study, the bilateral microinjection of U-50488H into the magnocellular PVN of ketamine/xylazine-anesthetized rats evoked a marked increase in urine flow rate that peaked approximately 30 to 40 min after drug injection and which returned to control levels after 70 min. Although microinjection of U-50488H into the magnocellular PVN increased urine flow rate, the diuretic response was not accompanied by a change in urinary sodium excretion or renal sympathetic nerve activity. This is in contrast to the concurrent diuretic, antinatriuretic and renal sympathoexcitatory responses produced by the i.c.v. injection of U-50488H observed in both ketamine/xylazine-anesthetized rats (Cabral et al., 1997 and present studies) and conscious rats (Kapusta and Obih, 1993; 1995). These findings indicate that exclusive activation of kappa opioid receptors in the magnocellular PVN has a selective action to control the renal excretion of water and produce diuresis. Although not tested, one premise is that the diuresis to U-50488H resulted from the inhibition of magnocellular secretory pathways for vasopressin which project to the posterior pituitary. In support of such a pathway, it has been demonstrated that by reducing the magnitude of depolarizing after-potentials, kappa opioids decrease post-spike excitability and

the duration of phasic bursts in vasopressin cells *in vivo* (Brown et al., 1999; Brown and Leng, 2000).

In contrast to hormonal regulation, the parvocellular division of the PVN has an established role in the control of central sympathetic outflow (Strack et al., 1989, Toney et al., 2003). Since i.c.v. administration of kappa opioid agonists produces antinatriuresis via a renal nerve pathway in which renal sympathetic nerve activity is augmented (Kapusta and Obih, 1993; 1995), in the present studies we sought to determine if kappa opioid systems change the renal excretion of sodium and/or water by altering sympathetic outflow to the kidneys from a locus within the PVN. This question was also of interest since there is evidence for an oligosynaptic projection from the PVN to the kidney, the activation of which can affect renal hemodynamics and the renal excretion of sodium via the renal sympathetic nerves (Schramm et al., 1993; Haselton and Vari, 1998).

Despite suggestive evidence, the results of this study indicate that kappa opioids do not act within the PVN to evoke the renal sympathoexcitatory and antinatriuretic responses characteristic of kappa opioids administered i.c.v. This conclusion is based on the following findings. First and foremost, the microinjection of U-50488H into either the magnocellular or parvocellular regions of the PVN failed to elicit an increase in renal sympathetic nerve activity or a decrease in urinary sodium excretion. In fact, when U-50488H was microinjected into the magnocellular division the level of renal sympathetic nerve activity remained unchanged throughout the duration of the solute free water diuresis. In contrast to these findings, microinjection of U-50488H into the parvocellular PVN did evoke significant changes in cardiovascular, renal excretory and renal sympathetic nerve activity, but these responses were qualitatively different and in opposition to those evoked by the i.c.v. injection of the same kappa

opioid in either ketamine/xylazine-anesthetized (Cabral et al., 1997; and current study) or conscious rats (Kapusta and Obih, 1993; 1995). In particular, in the present studies the microinjection of U-50488H into the parvocellular region (medial and lateral) of ketamine/xylazine-anesthetized rats produced an immediate, but transient pressor response that occurred together with reductions in heart rate and renal sympathetic nerve activity and an increase in urinary sodium excretion. When considering potential involvement of the renal nerves, the U-50488H-evoked decrease in renal sympathetic nerve activity observed in the current studies is not consistent with a neurally mediated antinatriuretic mechanism in which a renal sympathoexcitatory response would have been expected to occur. Thus, while central kappa opioid agonists have been demonstrated to produce antinatriuresis via augmenting sympathetic neural outflow to the kidneys, the findings of this study indicate that brain sites other than the PVN are involved in mediating the renal sympathoexcitatory response and antinatriuresis to central kappa opioids. We are currently investigating the role of other brain regions known to be involved in the control of central sympathetic outflow (e.g., rostral ventrolateral medulla, locus coeruleus, etc.) to determine whether kappa opioids may act in these sites to affect the renal tubular handling of sodium.

In the present investigations the mechanism(s) by which cardiovascular and renal function were altered following the microinjection of U-50488H into the parvocellular PVN was not studied. One possibility is that the pressor response evoked by U-50488H produced potentially baroreceptor reflex-evoked changes in heart rate (bradycardia), urinary sodium excretion (natriuresis) and renal sympathetic nerve activity (inhibitory). In regards to sodium excretion, it is also possible that the increase in mean arterial pressure elicited a pressure natriuresis. While the underlying neural mechanisms involved in mediating the pressor response also have yet to be

determined, it is worth noting that the pattern of cardiovascular and renal responses produced by activation of kappa opioid receptor pathways in the parvocellular PVN is unique. Thus, while low-level electrical or chemical (kainic acid, bicuculline) stimulation of the PVN has been shown to increase mean arterial pressure in anesthetized rats, in these instances the pressor response was associated with tachycardia and either, antinatriuresis and antidiuresis (Nelson et al., 1986; Haselton and Vari, 1998) or natriuresis and diuresis (Jin and Rockhold, 1989). Of interest, it has been shown that in anesthetized rabbits the primary pattern of responses produced by chemical (d,l-homocysteic acid) activation of the PVN is a pressor response (no change in heart rate) accompanied by a decrease in renal sympathetic nerve activity and an increase in splanchnic, adrenal, and cardiac sympathetic activity (Deering and Coote, 2000). Despite differences in experimental conditions (e.g., type of anesthetic, species, specific PVN subregion studied, mode of PVN activation/inhibition, etc.) which may contribute to the variations in cardiovascular, renal sympathetic nerve activity and renal excretory responses in these studies, it is evident that the PVN (particularly the paraventricular division) can play a pivotal role in regulating efferent sympathetic nerve discharge to different target organs under different physiological and potentially stressful/pathological conditions (see Kenney et al., 2003 for review).

In summary, the results of these studies demonstrate that microinjection of the selective kappa opioid receptor agonist, U-50488H, into subregions of the hypothalamic PVN produce differential cardiovascular and renal responses in ketamine/xylazine-anesthetized rats. In particular, microinjection of U-50488H into the magnocellular PVN produced a solute free water diuresis without altering systemic cardiovascular function or sympathetic outflow to the kidneys. Alternatively, activation of kappa opioid receptor pathways in the parvocellular PVN produced an increase in mean arterial pressure and urinary sodium excretion that was paralleled by a

decrease in heart rate and renal sympathetic nerve activity. Together, these findings demonstrate that the magnocellular PVN is an important brain site in which kappa opioids act to produce diuresis. However, it appears that central kappa opioids evoke antinatriuresis via augmenting central sympathetic outflow to the kidneys from a brain region outside of this hypothalamic nucleus.

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FIGURE LEGENDS

Figure 1. Cardiovascular and renal responses produced by i.c.v. U-50488H in ketamine/xylazine-anesthetized rats. Values are means ± S.E, illustrating the systemic cardiovascular and renal responses produced by i.c.v. administration of the selective kappa opioid agonist, U-50488H (●, 10 μg; n = 7) or isotonic saline vehicle (○, 5 μl; n = 6), in ketamine/xylazine-anesthetized rats. Consecutive 10-min urine samples were collected during control (C), and after i.c.v. drug injection (time points 10-80 min). HR, heart rate; MAP, mean arterial pressure; V, urine flow rate; UNaV, urinary sodium excretion; RSNA, renal sympathetic nerve activity (% of control).* $P < .05$, significantly different from respective group control value.

Figure 2. Cardiovascular and renal excretory dose-response effects produced by bilateral microinjection of U-50448H into the magnocellular region of the PVN in ketamine/xylazine-anesthetized rats. Values are means ± S.E, illustrating the systemic cardiovascular and renal excretory responses produced by bilateral microinjection of the kappa opioid agonist, U-50488H, into brain sites within (▼, 10 ng; n = 6; ●, 100 ng; n = 8) and outside (Δ, 100 ng; n = 5) of the magnocellular region of the PVN of ketamine/xylazine-anesthetized rats. Abbreviations are same as in Fig. 1. $*,^{t} P < .05$, significantly different from respective group control value.

Figure 3. Cardiovascular, renal excretory and renal sympathetic nerve responses produced by bilateral microinjection of U-50488H into the magnocellular region of the PVN of ketamine and xylazine-anesthetized rats. Values are means ± S.E. Cardiovascular, renal excretory and renal sympathetic nerve activity responses were measured during control (C) and experimental periods (time points 10-80 min) following microinjection of U-50488H into the magnocellular region of the PVN in naïve rats (●, 100 ng; n = 5) and rats pretreated (10-min) with the kappa receptor antagonist, nor-BNI (○, 100 ng, magnocellular PVN; n = 5). Responses produced by microinjection of U-50488H into regions outside (Δ, 100 ng; n = 6) of the PVN are also depicted. Abbreviations are same as in Fig. 1. RSNA, renal sympathetic nerve activity (% control). * P < .05, significantly different from respective group control value.

Figure 4. Cardiovascular and renal responses produced by bilateral microinjection of U-50488H into the parvocellular region of the PVN of ketamine and xylazine-anesthetized rats. Values are means ± S.E. Cardiovascular, renal excretory and renal sympathetic nerve activity responses were measured during control (C) and experimental periods (time points 10-80 min) following microinjection of U-50488H into the parvocellular region of the PVN in naïve rats (●, 100 ng; n = 5) and rats pretreated (10-min) with the kappa receptor antagonist, nor-BNI (○, 100 ng, parvocellular PVN; n = 6). Responses produced by microinjection of U-50488H into regions outside (Δ, 100 ng; n = 6; data same as that shown in Fig. 3) of the PVN are also depicted. Abbreviations same as in Figs 1 and 3. * P < .05, significantly different from respective group control value.

Figure 5. Histologically verified sites for which data are presented in Figs. 3 and 4 into which the selective kappa opioid receptor agonist, U-50488H (100 ng in 60 nl) was microinjected into the magnocellular (n = 5) or parvocellular regions of the PVN (n = 8), or regions outside of the PVN (n = 6).

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Fig. 1

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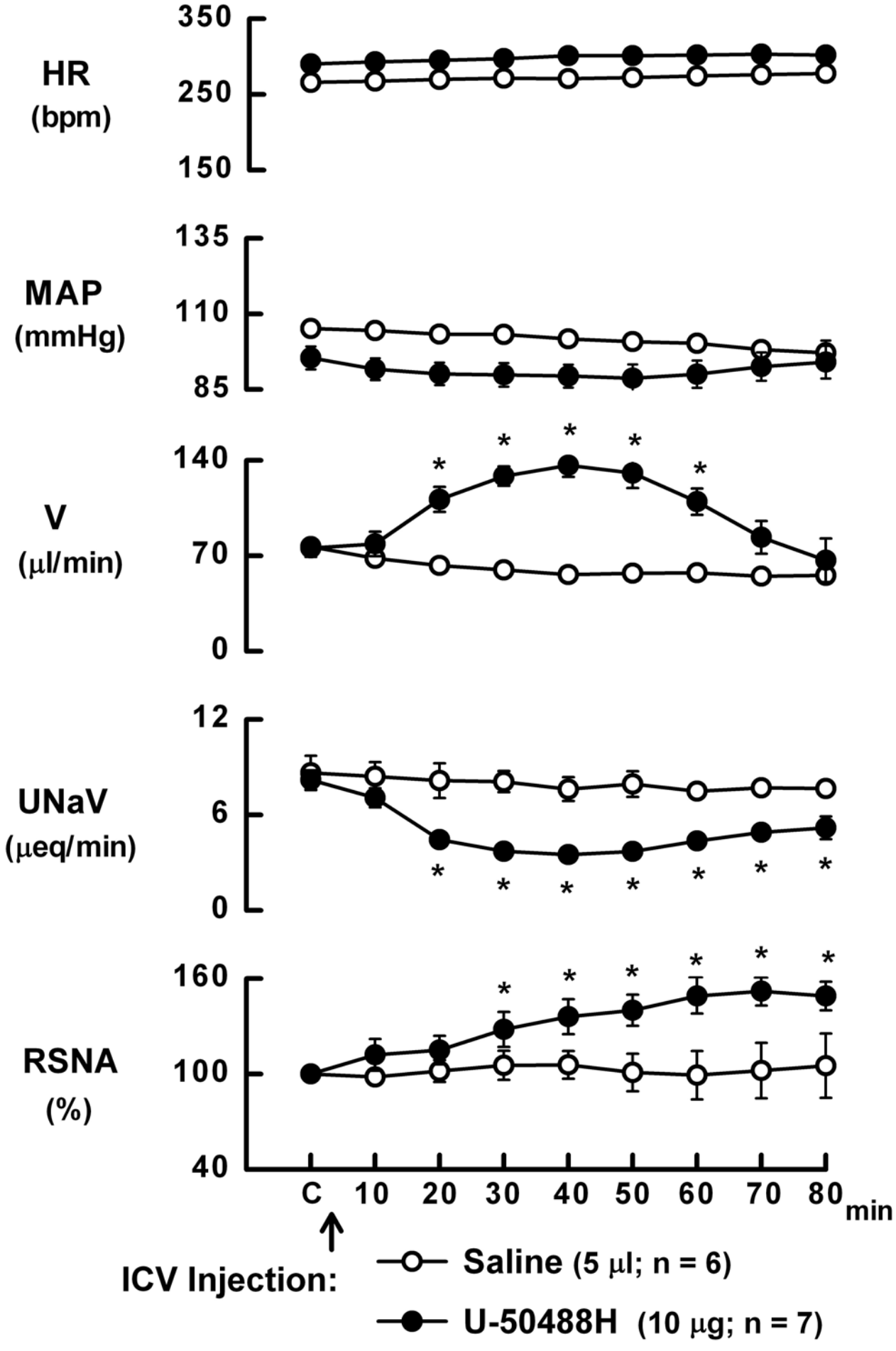

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Fig. 2

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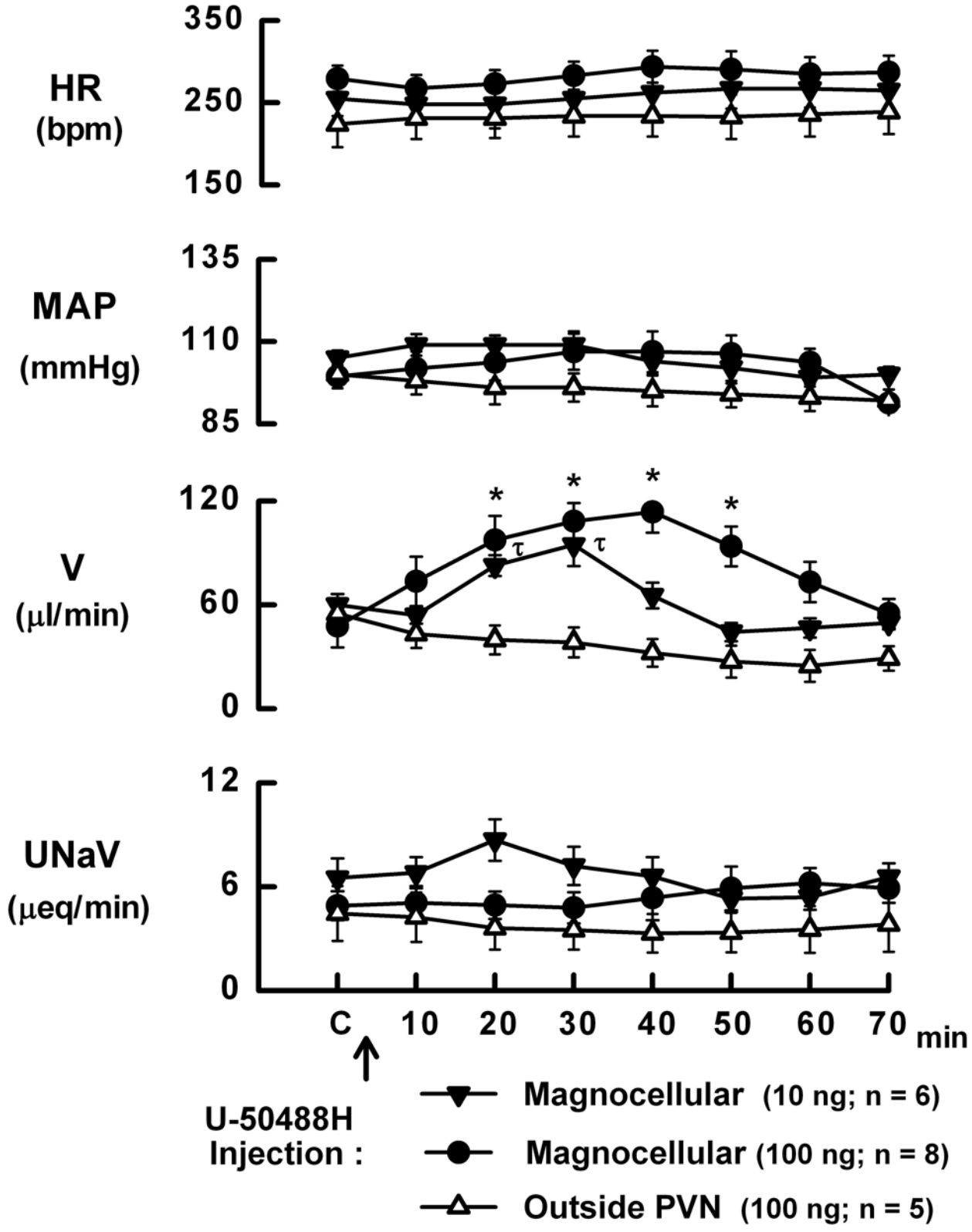

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Fig. 3

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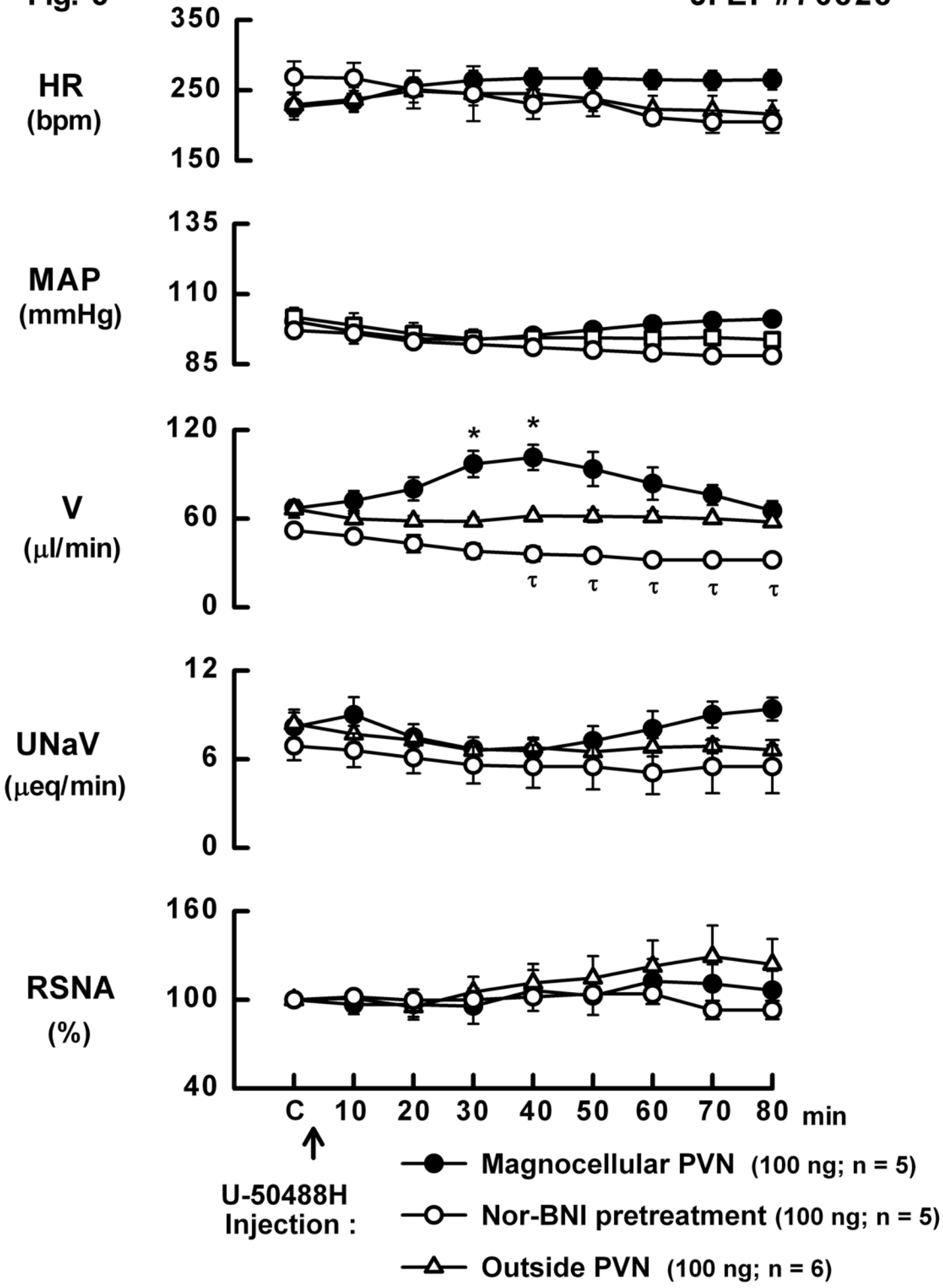

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Fig. 4

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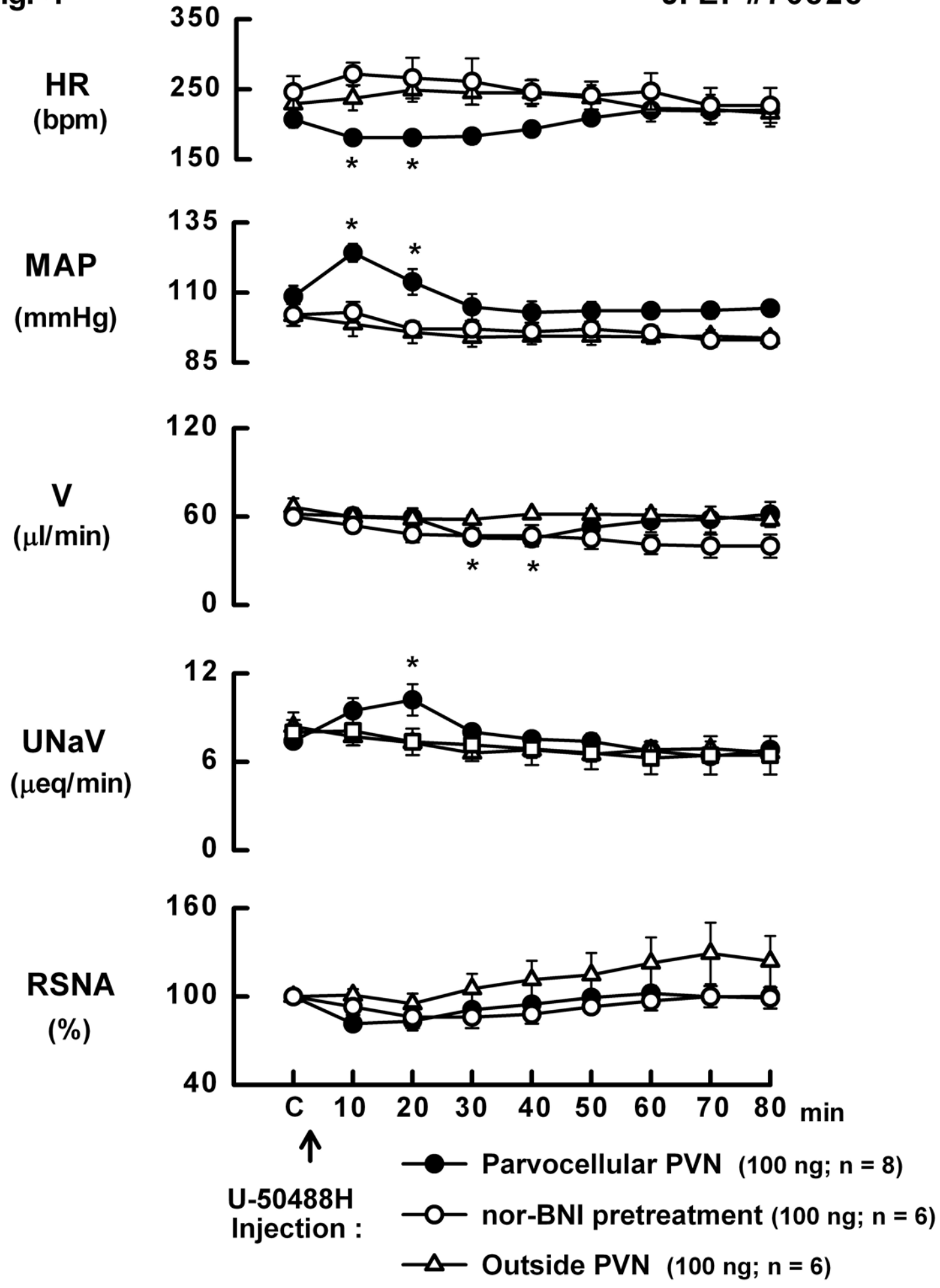

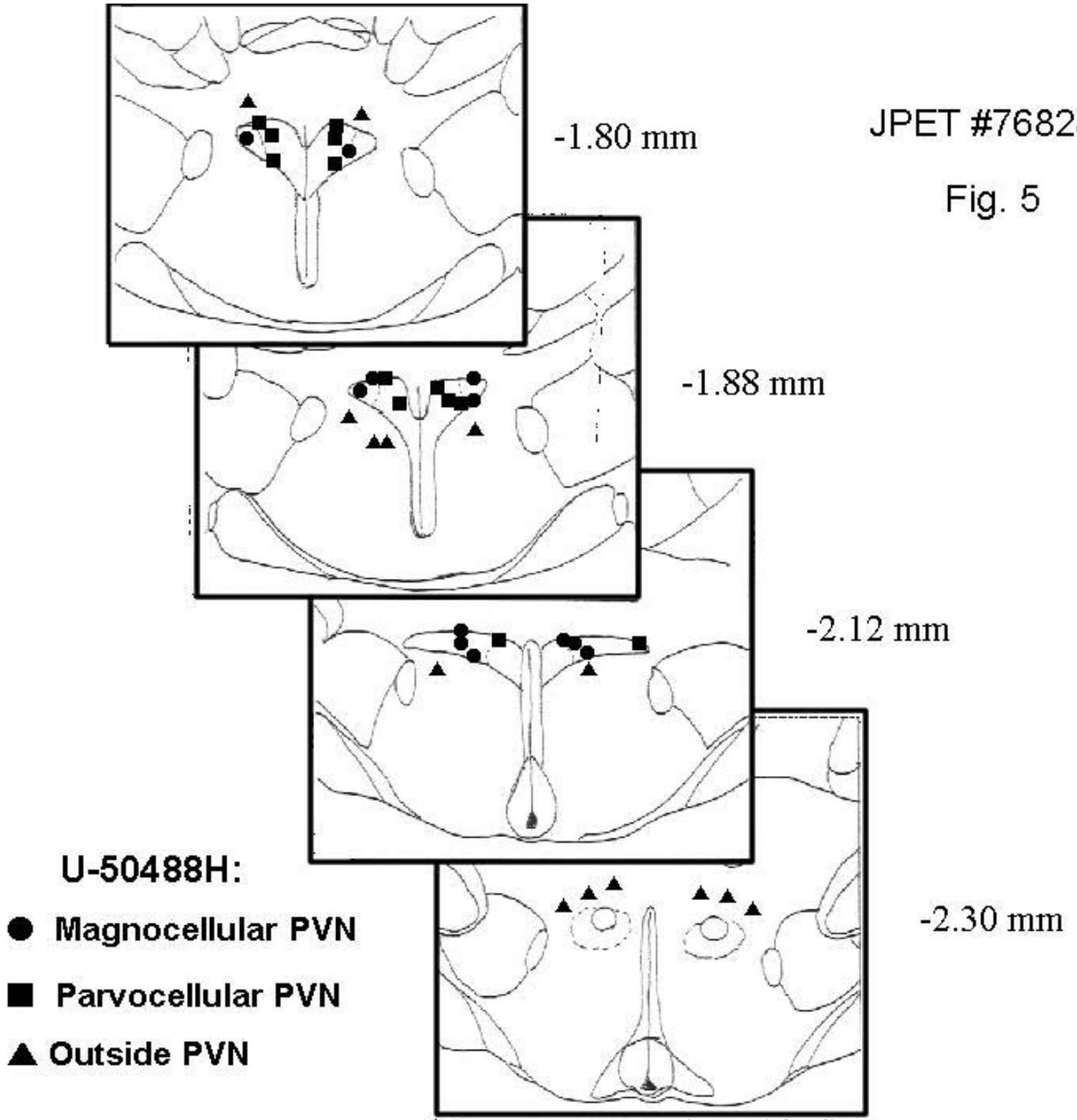

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Fig. 5