Urotensin II (UII) is a cyclic neuropeptide with strong vasoconstrictive activity in the peripheral vasculature. UII receptor mRNA is also expressed in the CNS, in particular in cholinergic neurons located in the mesopontine tegmental area, including the pedunculopontine tegmental (PPT) and lateral dorsal tegmental nuclei. This distribution suggests that the UII system is involved in functions regulated by acetylcholine, such as the sleep–wake cycle. Here, we tested the hypothesis that UII influences cholinergic PPT neuron activity and alters rapid eye movement (REM) sleep patterns in rats. Local administration of UII into the PPT nucleus increases REM sleep without inducing changes in the cortical blood flow. Intracerebroventricular injection of UII enhances both REM sleep and wakefulness and reduces slow-wave sleep 2. Intracerebroventricular, but not local, administration of UII increases cortical blood flow. Moreover, whole-cell recordings from rat-brain slices show that UII selectively excites cholinergic PPT neurons via an inward current and membrane depolarization that were accompanied by membrane conductance decreases. This effect does not depend on action potential generation or fast synaptic transmission because it persisted in the presence of TTX and antagonists of ionotropic glutamate, GABA, and glycine receptors. Collectively, these results suggest that UII plays a role in the regulation of REM sleep independently of its cerebrovascular actions by directly activating cholinergic brainstem neurons.

Key words: urotensin II; REM sleep; urotensin receptors; acetylcholine; pedunculopontine tegmental nucleus; laterodorsal tegmental nucleus; cortical blood flow

Introduction
Urotensin II (UII) is a neuropeptide that was initially isolated from urophysis extracts of several species of fish (Pearson et al., 1980), with a core structure that is conserved through mollusks to mammals (Conlon et al., 1996, 1997; Chartrel et al., 1998; Coulouarn et al., 1998, 1999). UII has potent vasoconstrictor activity in the periphery (Russell et al., 2001; Bohm and Pernow, 2002). However, intracerebroventricular injection or intra-arterial injection of UII induces hypotensive and bradycardiac effects in rats (Gibson et al., 1986), suggesting that central UII plays a role in cardiovascular homeostasis through its specific receptor in blood vessels and in the CNS (Ames et al., 1999; Liu et al., 1999; Mori et al., 1999; Nothacker et al., 1999). Recent studies have shown that UII receptor mRNA colocalizes with choline acetyltransferase in the mesopontine tegmental area, including the pedunculopontine tegmental (PPT) and the lateral dorsal tegmental (LDT) nuclei (Clark et al., 2001).

The distribution of UII receptor mRNA in the cholinergic PPT and LDT neurons suggests that, in addition to its vascular actions, the UII system may be involved in the regulation of the sleep–wake cycle. Previous studies in cats and rats have demonstrated the role of PPT and LDT nuclei in sleep regulation. The injection of cholinergic agonists into the medial pontine reticular formation, a target region of the PPT and LDT, induces a rapid eye movement (REM)-like state (George et al., 1964; Baghdoyan et al., 1984; Quattrochi et al., 1989; Bourgin et al., 1995) including cortical desynchronization, hippocampal theta rhythm, muscle atonia, reduction of REM sleep onset, and increase in total time of REM sleep (Vanni-Mercier et al., 1989; Yamamoto et al., 1990; Vertes et al., 1993). These and other studies (for review, see Steriade and McCarley, 1990) clearly indicate that cholinergic neurons in the PPT are important regulators of REM sleep.

Considering these studies and the observation that cholinergic PPT and LDT neurons express UII receptor mRNA, we tested the hypothesis that UII can significantly influence cholinergic PPT and LDT neuron activity and alter REM sleep patterns in rats. In addition, because UII induces changes in blood pressure (Gibson et al., 1986), and because it has been proposed that the hypnogenic properties of some putative sleep factors could be related to changes in blood pressure (Feinberg and Campbell, 1999), we also measured cortical blood flow (CBF) in animals treated with UII.

Our results show that both local injection of UII (0.6 pmol) into the PPT nucleus and intracerebroventricular administration of UII...
(0.6 nmol) dramatically increase the amount of REM sleep in rats. Relative cerebral blood flow was unaffected when UII was administered into the PPT nucleus. Furthermore, using whole-cell recordings, we demonstrate that UII selectively excites mesopontine cholinergic PPT neurons by activating a slow inward current. Collectively, our data strongly suggest that UII can function to modulate REM sleep by enhancing the excitability of mesopontine cholinergic neurons, independent of its effects on the cerebral vasculature.

Materials and Methods

Animals and surgery. Thirty-two adult male Sprague Dawley rats (250–300 g) were implanted under halothane anesthesia (1–2%), with a standard set of stainless-steel screw electrodes for chronic sleep recordings. The electroencephalogram (EEG) was recorded from electrodes placed on the frontal (two electrodes) and parietal (two electrodes) bone over the hippocampus [anteroposterior (AP), −2.46; mediolateral (ML), ±2.0]. A fifth EEG electrode was placed over the cerebellum to ground the animal and to reduce signal artifacts. Two wire electrodes were inserted in the neck musculature to record postural tone through electromyographic (EMG) activity. Insulated leads from the EEG and EMG electrodes were then soldered to a miniconnector that was cemented to the skull with dental acrylic. In addition to the EEG and EMG electrodes, a chronic guide stainless-steel cannula was implanted bilaterally in the pars dissipatus region of the PPT nucleus (AP, −0.0; ML, ±1.4; dorsoventral (DV), ±2.8). A second group of rats (n = 10) was implanted bilaterally in the pars dissipatus region of the PPT nucleus (AP, −8.0, ML, ±2.0; DV, −5.7) (Paxinos and Watson, 1986). A third cohort of rats (n = 8) was implanted bilaterally with guide cannulas into the PPT pars compacta (PPT-pc) region (AP, −8.3; ML, ±2.0; DV, −7.0), which has a dense population of cholinergic neurons and is the region in which the electrophysiological recordings were conducted. To evaluate the specificity of effects of UII on PPT cholinergic neurons, four rats were implanted with a chronic guide stainless-steel cannula into the right locus ceruleus (LC) (AP, −12.35; ML, ±1.1; DV, +7.45, using a posterior angle of 20° from vertical).

After surgical implantation and appropriate wound closure, rats were housed in individual Plexiglas recording cages placed in environmentally controlled chambers (Tech/Serv model EPC-100; BRS/LVE, Laurel, MD). The animals were allowed sufficient time to recover before the study. During anesthetic recovery, animals were observed in a clean single cage, and their normal righting capability and locomotion were monitored. The ambient temperature was maintained at 25°C ± 1, and a 12 h light/dark cycle was maintained throughout the 10 d recovery period and the subsequent experiment period. Food and water were available ad libitum.

Sleep recordings. Rats were housed in individual recording cages that were maintained at the same temperature and light/dark cycle described above. To record sleep–wake states, rats were connected to commutators with flexible cables, allowing their unrestricted movement within the cage, and they were habituated to the recording cages for 96 h. The EEG and EMG signals were amplified in a Grass Instruments (Quincy, MA) model 7D polygraph, filtered in a frequency range of 0.30–75 Hz and sampled at 256 Hz. The EEG and electromyogram were displayed on a computer monitor and stored with a resolution of 128 Hz for off-line scoring of sleep–wake states and spectral analysis, using software supplied by Kissei Comtec (Irvine, CA). The EEG and electromyogram were recorded over 6 h. The recording chambers contained a mini video camera for continuously observing animal behavior during the recording sessions.
Experimental protocol. Six days after surgery, the patency and free drainage of the guide cannula were assessed in animals intracerebroventricularly implanted by microinjection of 5.0 μl of pyrogen-free isotonic saline (PFS; Abbott Laboratories, North Chicago, IL) over a 5 min period. The patency for cannulas implanted into the PPT nucleus was assessed by microinjection of 0.5 μl of PFS over a 10 min period. Procedures for minimizing potential contamination by extraneous pyrogens were used for all drug solutions and vehicles.

The sleep recordings on rats implanted with an intracerebroventricular cannula began 5 h after the onset of the light period (circadian time (CT) 5; CT0, lights on) observing the following schedule: on day 1, rats were injected intracerebroventricularly with 5.0 μl of PFS vehicle at CT5, and recordings were continued for 6 h; on day 3, the same rats were challenged at CT5 with 0.6 nmol of UII in 5.0 μl of vehicle. Injections were made over a 5 min period. UII was obtained from Bachem (Torrance, CA).

The cohort of rats implanted with cannulas in the PPT nucleus pars dissipatus and four rats implanted with cannulas into the PPT-pc were recorded according to the following schedule: on the first day, rats received a bilateral injection of 0.5 μl of PFS vehicle into the PPT nucleus at CT5, and recordings were continued for 6 h; on the third and fifth days, rats were injected bilaterally at CT5 into the PPT nucleus with 0.6 and 6.0 pmol, respectively, of UII in 0.5 μl of vehicle.

The other four rats implanted with cannulas into the PPT-pc received, on day 1, a bilateral injection of 0.3 μl of PFS. On day 3, 5 ng of Cys-Pal-D-Trp-Lys-Val-Cys-Cpa-NH2 (SB-710411), a UII receptor antagonist (Benveniste et al., 2002, 2004), was injected and followed 30 min later by a 0.6 pmol injection of UII. On day 5, animals were treated with 3 ng of SB-710411.

For both intracerebroventricular and microinjection experiments, UII treatments were done at CT5, and 6 h recordings were obtained. We skipped 1 d between treatments to ensure that the effects of the previous treatment had disappeared. All microinjections were made over a 10 min period.

To determine the spread of intracellular injections, four animals were injected with 0.2 μl of biotinylated UII (Phoenix Pharmaceuticals, Belmont, CA) into the PPT. The rats were killed 2 h after injection under deep anesthesia with 5% halothane by intracardial perfusion with 4% paraformaldehyde in phosphate buffer. The brain was removed, sliced, and stained for NADPH diaphorase, which stains cholinergic neurons in the brain. To determine the spread of intracerebroventricular injections, a stainless-steel guide cannula was implanted aseptically into the right lateral ventricle (AP, −0.34; ML, +1.4; DV, +2.8), whereas for PPT injections stainless-steel guide cannulas were implanted bilaterally into the PPT nucleus (stereotaxic coordinates: AP, −8.0; ML, ±2.0; DV, +5.7).

Experimental protocol. All experiments started at CT5, observing the following schedule: CBF was measured for 25 min before and 185 min after administration of UII in all animals. UII was administered intracerebroventricularly (n = 5) at a 0.6 nmol dose (5 μl volume) and directly into the PPT nucleus (n = 5) at a 0.6 pmol dose (0.5 μl volume).

Monitoring CBF and data analysis. CBF values were observed every 5 min and expressed as a percentage increase relative to the resting baseline level. LD flowmetry monitors relative changes in cerebral blood flow (Dirnagl et al., 1989; Iadecola and Reis, 1990). To minimize confounding effects of anesthesia, the time interval between administration of halothane and testing of hippocampal blood flow was kept consistent. Data were compared by a repeated-measures ANOVA, followed by a Scheffé’s F test for specific comparisons when indicated.

In vitro studies
Slice and whole-cell recordings. Brain slices were prepared and whole-cell recordings were conducted as described previously in detail (Burlet et al., 2002). Briefly, 250 μm slices containing the PPT were prepared from isoflurane-anesthetized Sprague Dawley rats (postnatal days 12–20; Charles River Laboratories, Wilmington, MA) in ice-cold, carboxy-equilibrated, artificial CSF (ACSF), which contained the following (in mM): 121 NaCl, 5 KCl, 1.2 NaH2PO4, 2.7 CaCl2, 1.2 MgSO4, 26 NaHCO3, and 20 dextrose. Using a fixed-stage microscope (model BX50WI; Olympus, Tokyo, Japan), the boundaries of the PPT were first determined by low-magnification inspection, and then neurons to be recorded were visualized with a cooled CCD video camera (Dage 300F; Dage-MTI, Michigan City, IN) using a 40× water-immersion objective and infrared differential interference contrast (DIC) optics. The submerged slice recording chamber was perfused at 3–5 ml/min with room-temperature ACSF. In some experiments, 0.1% (by weight) BSA (Sigma, St. Louis, MO) was included in the ACSF. Rat urotensin II (Phoenix Pharmaceuticals) was dissolved in the perfusate (300 μl) just before the experiment and was applied by bath superfusion. In selected bridge-mode recordings aimed at evaluating presynaptic versus postsynaptic contributions to the UII response, tetrodotoxin (0.5 μM), DNPX (15 μM), APV (50 μM), bicuculline (10 μM), and strychnine (2.5 μM) were included in the superfusion media to block action potentials and fast synaptic potentials.

Giga-seal whole-cell voltage- and current-clamp recordings of PPT neurons were made with pipettes pulled from 1.5-mm-diameter glass capillary tubing (Corning 7052; A&M Systems, Everett, WA) using an Axopatch 200B amplifier (Molecular Devices, Union City, CA). The pipette solution contained the following (in mM): 140 K-glucocanate, 4
MgATP, 10 HEPES, 0.3 NaGTP, and 4 NaCl. Biocytin Alexa Fluor 594 (10 μM; Molecular Probes, Eugene, OR) was included in the patch solution for identification of recorded cells. Membrane voltages and currents were controlled and recorded with a computer running pClamp8 software (Molecular Devices). The quality of recordings was assessed by on-line monitoring of access resistance (<31 MΩ), input resistance, holding current, and capacitance. Recordings were terminated if the estimated access resistance became unstable or changed by >20% between measurements. Recordings were uncompensated for series resistance errors, because the recorded currents and associated voltage errors were small. Electrophysiological signals were filtered at 2 kHz and sampled at 5 kHz.

Immunocytochemistry. Cell identification was performed by immunocytochemistry as described previously in detail for brain nitric oxide synthase (bNOS) (Burlet et al., 2002), which is a convenient marker for cholinergic neurons in the LDT and PPT (Vincent and Kimura, 1992). Briefly, after successful removal of the patch pipette from the Alexa Fluor-filled neuron, the slice was fixed in 4% paraformaldehyde for 1–3 d at room temperature, cryoprotected by equilibration in 30% sucrose for 20% be NOS-immunoreactive; Sigma) and visualized with FITC-conjugated goat anti-rabbit and anti-mouse IgGs (1:50 in PBS; Chemicon, Temecula, CA) to determine whether recorded cells were bNOS-immunoreactive.

Peptides. Rat urotensin II was synthesized by The Scripps Research Institute peptide core facility or purchased from Phoenix Pharmaceuticals or Bachem. Peptide identity was confirmed by mass spectrometry. The UII receptor antagonist SB-710411 (Behm et al., 2002) and biotinylated UII were purchased from Phoenix Pharmaceuticals.

**Results**

**Effects of local administration of UII into the PPT nucleus**

Effects on the sleep–wake pattern and EEG spectra

We tested the effects on the sleep–wake cycle of local bilateral injections of 0.6 and 6.0 pmol of UII into the PPT nucleus. To locate the specific injection sites, we mapped them histologically according to the atlas of Swanson (1992) (Fig. 1). Administration of 0.6 pmol of UII induced a significant increase in REM sleep of 90.0, 59.0, and 69.8%, respectively, 2, 3, and 4 h after treatment, 

$F(3,290) = 4.35, p < 0.01; F(3,18) = 4.00, p < 0.05$; and

$F(3,18) = 6.19, p < 0.01, \text{ respectively}$, compared with PFS vehicle. This increase was blocked by pretreatment with SB-710411, a UII receptor antagonist (Behm et al., 2002). The increase in REM sleep was linked to a decrease in the amount of W during the third and fourth hours after the injection of UII; however, such effects were not significant (Fig. 2). The increase in REM sleep was attributable to a significant increment of 45.3% ($F(3,18) = 3.11, p < 0.05$) in the mean number of REM sleep episodes, whereas the UII receptor antagonist blocked this effect (Table 1). Administration of 0.6 pmol of UII into the PPT nucleus did not produce any significant change in the amount of SWS1 and SWS2. Likewise, no significant changes in any of the stages of vigilance or sleep parameters were observed when animals were treated with 6.0 pmol of UII (Fig. 2). Similar results were observed in rats with cannulas implanted into the PPT-pc, and no significant differences were observed between animals with cannulas implanted into the PPT pars dissipatus and those with cannulas implanted into the PPT-pc (data not shown).

UII administration (0.6 pmol) led to marked changes in the EEG power density in some frequency bands compared with saline and 6.0 pmol of UII. An increase in the power was observed both in $\gamma (F(2,2900) = 17.10; p < 0.01)$ and $\theta (F(2,2900) = 10.58; p < 0.01)$ frequency bands during W and REM sleep, respectively, whereas no significant changes were observed with 6.0 pmol of UII. These increases in EEG power density were blocked by the SB-710411 (Fig. 3).

Administration of UII into the LC produced no significant change in the amount of W (125.94 ± 12.58 min), SWS1 (9.81 ± 1.0 min), SWS2 (197.19 ± 9.51 min), and REM sleep (27.12 ± 5.45 min) compared with saline administration (W, 111.63 ± 3.52; SWS1, 10.38 ± 2.03; SWS2, 200.25 ± 4.31; REM sleep, 37.87 ± 3.59 min). Significant differences between groups were not observed in the sleep parameters or in the EEG power spectra (data not shown).

Effects on cortical blood flow

Administration of 0.6 pmol of UII into the PPT nucleus did not induce major changes in cortical blood flow compared with
The amount of SWS2 was significantly decreased 1 and 2 h after injection of 0.6 nmol of UII induced a significant increase of 78.7, 108.1, and 108.8% in the amount of REM sleep 2, 3, and 5 h after treatment, respectively ($F_{1,10} = 24.68$, $p < 0.001$; $F_{1,10} = 8.21$, $p < 0.01$; and $F_{1,10} = 11.19$, $p < 0.01$, respectively) compared with saline injections (Fig. 5). No significant differences were observed in the amount of SWS1 after the administration of UII. The effects on REM sleep after intracerebroventricular administration of UII were attributable to an increase of 85.2% in the amount of REM sleep 2, 3, and 5 h after injection ($F_{1,10} = 5.73$, $p < 0.05$) (Fig. 4A).

**Effects of intracerebroventricular administration of UII**

**Effects on the sleep–wake patterns and EEG spectra**

To test whether intracerebroventricular administration of UII induces changes in sleep–wake patterns, we challenged the animals with 0.6 nmol of UII. UII induced a significant increase of 37.0% in the total amount of W during the first hour after treatment, compared with saline administration ($F_{1,10} = 5.574; p < 0.05$). The amount of SWS2 was significantly decreased 1 and 2 h after UII injection (53.3 and 20.0%, respectively; $F_{1,10} = 12.84$, $p < 0.01$; $F_{1,10} = 14.68$, $p < 0.01$, respectively), compared with saline administration. Likewise, intracerebroventricular administration of 0.6 nmol of UII induced a significant increase of 78.7,
current (−11.8 ± 1.9 pA; mean ± SEM) (Fig. 7A) in 11 of 15 PPT neurons, which was accompanied by a 35% increase in input resistance [from 241 ± 28 to 324 ± 36 MΩ (±SEM)]. These actions were specific for cholinergic neurons (p = 0.009; Fisher’s test), because UII evoked such an inward current in 11 of 12 bNOS-immunopositive neurons, whereas UII failed to have an effect on three of three bNOS-immunonegative PPT neurons (Fig. 7B).

As expected, current-clamp recordings revealed that UII produced membrane depolarization and an increase in firing of PPT neurons (n = 6 of 8; 4 of 6 confirmed bNOS-immunopositive). An example of the ability of UII to alter spike discharge rate is shown in Figure 8A, in which the UII-evoked depolarization drives a quiescent neuron into prolonged repetitive firing. The amplitude of the membrane depolarization elicited by UII was estimated to be 5.7 ± 1.2 mV in neurons held at subthreshold potentials (n = 3). After blockade of action potentials and fast synaptic activity with TTX, APV, DNQX, strychnine, and bicuculline, UII still evoked comparable depolarizations (7.0 ± 0.6 mV; n = 3; all bNOS-immunopositive) (for sample response, see Fig. 8B), suggesting a postsynaptic site of action. As observed in voltage-clamp recordings, membrane input resistance also increased during these UII-evoked depolarizations (Fig. 8C). Finally, in the time frame of our recording experiments, a second action of UII only produced responses that were greatly attenuated, consistent with desensitization. Collectively, data from whole-cell recordings strongly suggest that UII selectively and directly excites cholinergic PPT neurons by activating a slow inward current.

Discussion

We hypothesized that the UII system could be involved in the regulation of REM sleep based on the fact that UII receptors colocalize with ChAT in the mesopontine tegmental area, including PPT and LDT nuclei (Clark et al., 2001). Our results show that both local PPT and intracerebroventricular administration of low doses of UII dramatically increased the total time of REM sleep in rats. This effect could be accounted for by a significant increase in the number of REM episodes. Likewise, our results showed that UII selectively excites PPT cholinergic neurons at their resting membrane potential by activating a slow inward current (Fig. 7), supporting the hypothesis that increments in REM sleep caused by UII could be a result of neuroexcitation of mesopontine cholinergic neurons. In addition, the absence of a UII-related increase in REM sleep in both animals pretreated with SB-710411 and rats treated with UII in the LC strongly suggests that UII regulates REM sleep through activation of UII receptors located in PPT neurons and that its effects are not mediated through metabolic products.

Both the LDT and PPT send cholinergic projections to the REM sleep, generating neurons located in the pontine reticular formation (Mitani et al., 1988). These inputs, when stimulated, produce EPSPs in the pontine reticular formation neurons (Imon et al., 1996; Homma et al., 2002). Because of the stimulatory effect of UII on PPT neurons, it is likely that UII induces acetylcholine release in the pontine reticular formation and induces REM sleep. The magnitude of the increase in REM recorded in this study is at least as large as that recorded when carbachol is injected into the oral pontine reticular nucleus of the rat, which induces an increase in REM sleep episodes with no change in episode length or latency to REM onset (Bourgin et al., 1995). Microinjection of carbachol into the medial gigantocellular tegmental field produces an increase in ACh release for 7 h that has been related to
PPT/LDT activation (Lydic et al., 1991; Lydic and Baghdoyan, 1993), whereas glutamatergic stimulation of PPT increases REM sleep up to 6 h after injection (Datta et al., 2001). These results mirror the parameters of the UII-induced increase in REM state. The slow onset of the UII-induced increase in REM sleep in our studies could be caused by the slow diffusion rate of UII in the brain parenchyma, as described previously for hypocretin (Hcrt) (Vertes et al., 1993; Steriade et al., 1993).

Another key finding of this study was that UII excited bNOS-immunopositive PPT neurons by activating a slow inward current. Blocking action potential discharge and antagonizing fast glutamatergic thalamic afferents produces cortical activation by suppressing slow cortical waves between 0.3 and 4.0 Hz and spindle wave oscillations (11.0–14.0 Hz) (Hu et al., 1989; Curro Dossi et al., 1991; Steriade et al., 1993).
did not attenuate the response to UII. This is consistent with a direct postsynaptic action of UII and, considering our immunocytochemical findings, implies that the functional UII receptors reside on cholinergic PPT neurons.

We also found that input resistance increased during UII-evoked excitation in both voltage-clamp and bridge-mode recordings. This suggests that UII acts by blocking a hyperpolarizing conductance, which is activated around the resting potential, e.g., a potassium current. Additional work will be necessary to identify the underlying current(s). Nevertheless, this elevation of input resistance should also enhance the effectiveness of synaptic inputs, which may further increase PPT neuron excitation.

The UII-evoked increase in PPT neuron discharge rate will also promote the release of ACh at PPT target structures, such as the thalamus and reticular formation. Indeed, UII may have a general role in promoting ACh release, because, in the periphery, UII also promotes the release of ACh at PPT target structures, such as the paraventricular nucleus, arcuate nucleus, and noradrenergic cells in the inner part of the medulla induces cardiovascular effects (Lu et al., 2002). Thus, UII may have a neurogenic role in the regulation of cardiovascular physiology, and this may be the cause of the intracerebroventricular application-induced blood flow effects shown here. Despite these findings, an increase in cortical blood flow is not seen with local injections into the PPT. It is, therefore, likely that the blood flow increases are not a result of mesopontine cholinergic activation and would be a result of UII acting on noncholinergic neuronal sites (Lu et al., 2002). However, additional studies are needed to confirm that UII does not induce changes in local blood flow.

Together, the results are consistent with a role for UII in the regulation of REM sleep by activating mesopontine cholinergic neurons directly and independently of the cardiovascular effects of UII.

References


Burlet S, Tyler CJ, Leonard CS (2002) Direct and indirect excitation of lat-


yn Schmiedebers Arch Pharmacol 365:141–149.


bunda. Biochemical characterization and immunohistochemical localiza- 


Clark SD, Nothacker HP, Wang Z, Saito Y, Leslie FM, Civelli O (2001) The urotensin II receptor is expressed in the cholinergic mesopontine tegmen- 


transporter expression in the pons and medulla oblongata of the rat: 

localisation to noradrenergic and some 5 Ct adrenergic neurones. Brain 


forms of urotensin II and its role in cardiovascular regulation in verte- 


Regul Pept 69:95–103.

Couluar Y, Lührmann I, Jegou S, Anouar Y, Tostivint H, Beauvillain JC, 


the urotensin II precursor in frog and human reveals intense expression of 

the urotensin II gene in motoneurons of the spinal cord. Proc Natl Acad 

Sci USA 95:15803–15808.


sequence analysis and tissue distribution of the mouse and rat urotensin II 


Cravatt BF, Prospero-Garcia O, Siuzdak G, Henriksen SJ, Boger DL, 

Russell FD, Molenaar P, O'Brien DM (2001) Cardiostimulant effects of 


Quattrochi JJ, Mamelak AN, Madison RD, Macklis JD, Hobson JA (1989) 

Mapping neuronal inputs to REM sleep induction sites with carbachol- 

ergic projections from the laterodorsal and pedunculopontine teg-


Iadecola C (1997) Principles and methods for measurement of cerebral 

blood flow: experimental methods. In: Primer on cerebrovascular dis-

Eadecola C, Reis DJ (1990) Continuous monitoring of cerebrocortical blood 


Imon H, Ito K, Dauphin L, McCrory RW (1996) Electrical stimulation of 

the cholinergic laterodorsal tegmental nucleus elicits scopolamine-

sensitive excitatory postsynaptic potentials in medial pontine reticular 


the urethane anesthetized rat produce hippocampal theta rhythm and corti-

cal desynchronization: a comparison of pedunculopontine tegmental ver-


acid receptors in the paraventricular hypothalamic nucleus mediate pres- 

sor response induced by carotid body chemoreceptor stimulation in rats. 


Lerner RA, Siuzdak G, Prospero-Garcia O, Henriksen SJ, Boger DL, Cravatt 


Validation of laser-Doppler flowmetry in measurement of spinal cord 


Liu Q, Pong SS, Zeng Z, Zhang Q, Howard AD, Williams Jr DL, Davidoff M, 

Wang R, Austin CP, McDonald TP, Bai C, George SR, Evans JF, Caskey 

CT (1999) Identification of urotensin II as the endogenous ligand for 

the orphan G-protein-coupled receptor GPR14. Biochem Biophys Res 


in different brain areas. Peptides 23:1631–1635.

Lydic R, Baghdoyan HA (1993) Pedunculopontine stimulation alters respi- 

ration and increases ACh release in the pontine reticular formation. Am J 

Physiol 264:R544–554.


enhanced acetycholine release during state-dependent respiratory de-


Martin DS, Haywood JR (1992) Sympathetic nervous system activation by 


Mendelson WB, Bergmann BM (1999) EEG delta power during sleep in 


Cholinergic projections from the laterodorsal and pedunculopontine teg-

mental nuclei to the pontine gigantocellular tegmental field in the cat. 


Mori M, Sugo T, Abe M, Shimomura Y, Kurihara M, Kitada C, Kikuchi K, 

Shintani Y, Kurokawa T, Onda H, Nishimura O, Fujino M (1999) Uro-

tensin II is the endogenous ligand of a G-protein-coupled orphan recep- 


Muir JK, Boerschel M, Ellis EF (1992) Continuous monitoring of posttrau- 

matic cerebral blood flow using laser-Doppler flowmetry. J Neurotrauma 


SP, Civelli O (1999) Identification of the natural ligand of an orphan 

G-protein-coupled receptor involved in the regulation of vasoconstric-


into the pedunculopontine tegmental nucleus suppresses hippocampal 


San Diego: Academic.

Paxinos GWC, Watson AJ, Missler M, Schmahmann JD, Rottenberg D, 


Pearson D, Shively JE, Clark BR, Geschwind H, Barkley M, Nishioka RS, Bern 

HA (1980) Urotensin II: a somatostatin-like peptide in the caudal neu-


Quattrochi JJ, Mamelen AM, Madison RD, Macklis JD, Hobson JA (1989) 

Mapping neuronal inputs to REM sleep induction sites with carbachol-


Russell FD, Molenaar P, O'Brien DM (2001) Cardiostimulant effects of 


