Urethane inhibits the GABAergic neurotransmission in the nucleus of the solitary tract of rat brain stem slices

Daniela Accorsi-Mendonça, Ricardo M. Leão, José F. Aguiar, Wamberto A. Varanda, and Benedito H. Machado
Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
Submitted 2 November 2005; accepted in final form 24 August 2006

Urethane inhibits the GABAergic neurotransmission in the nucleus of the solitary tract of rat brain stem slices. Am J Physiol Regul Integr Comp Physiol 292: R396–R402, 2007. First published August 31, 2006; doi:10.1152/ajpregu.00776.2005.—Because urethane is a widely used anesthetic in animal experimentation, in the present study, we evaluated its effects on neurons of the nucleus of the solitary tract (NTS) in brain stem slices from young rats (25–30 days old). Using the whole cell configuration of the patch-clamp technique, spontaneous postsynaptic currents (sPSCs) and evoked excitatory postsynaptic currents (eEPSCs) were recorded. Urethane (20 mM) decreased by ~60% the frequency of GABAergic sPSCs (1.0 ± 0.2 vs. 0.4 ± 0.1 Hz) but did not change the frequency, amplitude, or half-width of glutamatergic events or TTX-resistant inhibitory sPSCs [miniature inhibitory postsynaptic currents (IPSCs)]. Miniature IPSCs were measured in the presence of urethane plus 1 mM diazepam (1 mM), and no changes were seen in their amplitude. The results suggest that the GABA concentration in the NTS synapses is set at saturating level. We also evaluated the effect of urethane on eEPSCs, and no significant change was observed in the amplitude of N-methyl-D-aspartate (NMDA; 44.2 ± 11.5 vs. 37.6 ± 10.6 pA [holding potential = 40 mV]) and non-NMDA currents [204.4 ± 35.5 vs. 196.6 ± 31.2 pA (holding potential = −70 mV)]. Current-clamp experiments showed that urethane did not alter the action potential characteristics and passive membrane properties. These data suggest that urethane has an inhibitory effect on GABAergic neurons in the NTS but does not change the spontaneous or evoked excitatory responses.

patch-clamp; slice; γ-aminobutyric acid current; neurotransmission; urethane

THE NUCLEUS OF THE SOLITARY TRACT (NTS), located in the dorsomedial aspect of the medulla oblongata, plays a key role in the control of several autonomic reflex functions, including respiratory, cardiovascular, gustatory, and gastrointestinal responses, because the afferents of these reflexes make their first synapse at this level of the central nervous system (CNS). Therefore, the NTS is an important integrative center of convergent information, playing a critical role in the modulation of the efferent autonomic responses (3, 24).

Microinjections of substances in the NTS have been used to infer functional properties of this nucleus, particularly those concerned with neurotransmission. Because of the small size and caudal location of NTS, this type of experiment is very sensitive to head and neck movements. For these reasons, the large majority of studies exploring this nucleus have been performed in animals under anesthesia.

Urethane (ethyl carbamate) is one of the most frequently employed anesthetics in studies related to cardiovascular research, since it produces a long-lasting surgical level of anesthesia (18, 19). Nevertheless, studies (17, 28) have shown that urethane has profound effects on the cardiovascular reflex responses by affecting neurotransmission in the brain stem. The lumbar sympathetic nerve activity, pressor response, and reflex excitation of the reticulospinal vasomotor neurons, elicited by stimulating the chemoreflex, were dose-dependently abolished by systemic administration of urethane (28). Previous results from our laboratory have shown that microinjection of L-glutamate in the NTS of awake rats produces a large increase in the mean arterial pressure (MAP), whereas in the same rat under urethane anesthesia, the microinjection of the same dose of L-glutamate leads to a fall in MAP (17).

Moreover, urethane affects the responses induced by neurotransmitter-gated ion channel receptors, potentiated the functions of neuronal nicotinic ACh, GABA_A, and glycine receptors, and inhibited N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazo leprropionic acid (AMPA) responses of receptors expressed in Xenopus oocytes (13). Study by Scholfield (26) also showed that urethane was the least potent of a series of anesthetics in producing enhancement of the inhibitory postsynaptic potential in guinea pig olfactory cortex, a response that involves a GABAergic mechanism.

In spite of urethane’s widespread use in animal research, there are few reports concerning its cellular actions. Nevertheless, the understanding of urethane’s mechanism of action upon the central neural pathways, particularly those involved in the cardiovascular function, is critically important for assessing the physiological relevance of data obtained under this type of anesthesia. Considering that the NTS plays a key role in the processing of several reflex afferents, the aim of the present study was to analyze the effect of acute application of urethane on spontaneous (excitatory and inhibitory) and excitatory-evoked synaptic neurotransmission in the NTS neurons in brain stem slices of the rat.

MATERIALS AND METHODS

All experimental protocols used in this work were reviewed and approved by the Institutional Ethical Committee for Animal Experimentation of the School of Medicine of Ribeirão Preto-University of São Paulo.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: B. H. Machado, Dept. of Physiology, School of Medicine of Ribeirão Preto, Univ. of São Paulo, Av. Bandeirantes, 3900, 14049-900 Ribeirão Preto, SP, Brazil (e-mail: bhmached@fmrp.usp.br).
Brain stem slices preparation. Male Wistar rats (25–30 days old) were decapitated without anesthesia, and the brain stem was rapidly removed and submerged in ice-cold (4°C) artificial cerebrospinal fluid (aCSF) containing (in mM): 120 NaCl, 2.5 KCl, 1 MgCl₂, 1.25 NaH₂PO₄, 25 NaHCO₃, 10 glucose, and 2 CaCl₂, with osmolality of 300–310 mosmol/kg H₂O and pH 7.4 when bubbled with 95% O₂ and 5% CO₂. Brain stem transversal slices (250 μm thick) were cut using an oscillating slicer (Vibratome 1000; Vibratome, St. Louis, MO) and kept in aCSF at 37°C for 45 min. A single slice was placed in the recording chamber, held in place with a nylon mesh, and continuously perfused with oxygenated aCSF at a rate of ~3 ml/min at room temperature (23–25°C).

Whole cell patch-clamp recordings. Whole cell recordings were made with patch pipettes pulled from thick-walled borosilicate glass capillaries (Sutter Instruments, Novato, CA), using a P-97 puller (Sutter Instruments). The resistance of the electrodes was 4–8 MΩ. For most of the experiments, patch pipettes were filled with an internal solution containing (in mM): 115 potassium gluconate, 10 KCl, 2 MgCl₂, 3 K-ATP, 10 EGTA, and 10 HEPES, with pH 7.3 adjusted with KOH. Under this condition, the calculated junction potential was equal to 15 mV, and the measurements were corrected accordingly. In the experiments where NMDA currents (AMPA/kainate currents) and at 0.03 Hz for eliciting NMDA currents (AMPA/kainate currents) were recorded in the current-clamp mode of the amplifier by applying a step-positive command potential, the internal solution contained (in mM): 115 potassium gluconate, 20 KCl, 3 K-ATP, 10 EGTA, and 10 HEPES, with pH 7.3 adjusted with KOH. All internal solutions had osmolality of 290–295 mosmol/kg H₂O and pH 7.4 when bubbled with 95% O₂ and 5% CO₂. Brain stem transversal slices (250 μm thick) were cut using a six-valve solenoid system (VC-6; Warner Instruments, Hamden, CT) and applied to the slices by a gravity-driven perfusion system (flow rate of 2–3 ml/min). Recordings started at least 2 min after initiating the drug perfusion.

The urethane concentration (20 mM) used in most of the experiments corresponds to the plasma concentration obtained during surgical anesthesia (18, 19, 28); however, we also tested 60 mM urethane. Considering that the results were similar using both concentrations, most of the presented data were obtained with the concentration of 20 mM.¹

RESULTS

Urethane inhibits the spontaneous synaptic transmission in the NTS. The acute application of 20 mM urethane affected the sPSCs (data not shown), significantly reducing the frequency of sPSCs by 45%, from 31.8 ± 0.9 to 1.4 ± 0.4 Hz (n = 8), without altering the amplitude (control: 31.8 ± 5.2 pA; urethane: 30.8 ± 4.5 pA) and the half-width of sPSCs (control: 5.1 ± 0.1 ms; urethane: 5.7 ± 0.6 ms). A higher concentration of urethane (60 mM) produced effects similar to 20 mM urethane; it also reduced the frequency of sPSCs from 7.3 ± 0.4 to 3.3 ± 1.4 Hz, without altering the amplitude (control: 45.9 ± 9 pA; urethane: 42.8 ± 13.3 pA) and the half-width of sPSCs (control: 5.0 ± 0.7 ms; urethane: 5.6 ± 0.9 ms, n = 8).

Considering that only glutamatergic and GABAergic spontaneous currents are reported to be present in neurons of NTS (6, 14, 23), we pharmacologically isolated spontaneous excitatory and inhibitory postsynaptic currents using the specific antagonists of non-NMDA and GABA_α receptors (DNQX and bicuculline, respectively) and studied the effect of urethane on both types of current.

Urethane inhibits sIPSCs. The sPSCs were isolated by perfusing the slice with aCSF containing 10 μM DNQX to block non-NMDA receptors. Figure 1A shows a typical trace of sIPSCs of an NTS neuron in the absence and presence of urethane. The distribution of frequency and amplitude of these events recorded from a representative neuron are shown in Fig. 1, B and C. Figure 1 also shows that urethane (20 mM) reduced the frequency of sIPSCs in 60% (sIPSCs: 1.0 ± 0.2 Hz; urethane: 0.4 ± 0.1 Hz, P < 0.05; Fig. 1D) but did not change the mean amplitude (sIPSCs: 30.3 ± 3.1 pA, urethane: 33.1 ± 8.2 pA, Fig. 1E) or the mean half-width (sIPSCs: 5.9 ± 0.5 ms; urethane: 5.2 ± 0.9 ms, Fig. 1F) of the events (n = 8).

¹ Although the osmolality of the extracellular solutions increased after addition of urethane (318 mosmol/kg H₂O after 20 mM urethane and 384 mosmol/kg H₂O after 60 mM urethane), there are no significant osmotic effects, since it diffuses through cell membranes, i.e., the membrane reflection coefficient (r) for urethane is smaller than one. This can be easily demonstrated by incubating red blood cells with hyperosmotic solutions of urethane and observing immediate hemolysis. Obviously, the same effect is being assumed for neural cells.
**Urethane does not affect sEPSCs.** The sEPSCs were isolated by perfusing the slice with aCSF containing 20 μM bicuculline, a GABA_A receptor antagonist. Figure 2 shows that urethane did not affect the frequency (sEPSCs: 0.5 ± 0.1 Hz; urethane: 0.4 ± 0.1 Hz; Fig. 2A), the mean amplitude (sEPSCs: 22.5 ± 1.6 pA; urethane: 22.6 ± 1.9 pA; Fig. 2B), or the mean half-width (sEPSCs: 3.0 ± 0.4 ms; urethane: 3.1 ± 0.6 ms; Fig. 2C) of the sEPSCs (n = 6).

**Urethane does not affect the miniature sIPSCs.** Because urethane affected only the frequency and not the amplitude of the sIPSCs, we can assume that it is acting presynaptically. We hypothesized that this anesthetic is acting by lowering the probability of firing of the GABAergic interneurons, and not on the release probability of a GABAergic vesicle, since the mean amplitude of the events was not altered by urethane. To confirm this hypothesis, we tested the effect of urethane on the action potential-independent events, i.e., the miniature GABAergic synaptic events (mIPSCs). In these experiments, the mIPSCs were recorded in the presence of 10 μM TTX (a Na^+^-channel blocker) and 10 μM DNQX. Consistent with our hypothesis, Fig. 3 shows that urethane did not produce any significant change in the mean frequency (TTX + DNQX: 0.6 ± 0.1 Hz; TTX + DNQX + urethane: 0.6 ± 0.2 Hz; Fig. 3A), amplitude (TTX + DNQX: 31.3 ± 2.1 pA; TTX + DNQX + urethane: 27.6 ± 2.7 pA; Fig. 3B), or half-width of the mIPSCs (TTX + DNQX: 5.5 ± 1.1 ms; TTX + DNQX + urethane: 5.9 ± 1.6 ms; Fig. 3C; n = 8). These results confirm that urethane is not acting on GABA_A receptor at the postsynaptic level and also that urethane does not change the release probability of a GABAergic vesicle.

**Diazepam does not affect the miniature sIPSCs in the presence of urethane.** It has been proposed that urethane increases the affinity of the GABA_A receptor to GABA with a conse-

---

*R398 SPONTANEOUS GABAERGIC CURRENTS ARE INHIBITED BY URETHANE*

AJP-Regul Integr Comp Physiol • VOL 292 • JANUARY 2007 • www.ajpregu.org

---

**Fig. 1.** A: representative traces of spontaneous inhibitory postsynaptic currents (sIPSCs) in a nucleus of the solitary tract (NTS) neuron in the presence of 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX) and DNQX + urethane (20 mM) for 5 min (holding potential = −70 mV). B: frequency distribution of these events in the presence of DNQX and DNQX + urethane (P < 0.0001, Kolmogorov-Smirnov test). C: amplitude distribution of the events in the presence of DNQX and DNQX + urethane (P = 0.07, Kolmogorov-Smirnov test). Summary of the effects of urethane (20 mM) on the frequency (D), amplitude (E), and half-width (F) of GABAergic sIPSCs in NTS neurons. *P < 0.05, Wilcoxon matched-pairs test (n = 8).
quent increase in the amplitude of the events (13); nevertheless, an increase on the mIPSC amplitude in the presence of urethane will only occur if the concentration of synaptic-released GABA does not saturate the postsynaptic receptors (22). To verify whether or not the synaptic-released GABA is saturating the NTS postsynaptic receptors, we compared the effect of urethane on the GABA mIPSCs in the absence and in the presence of 1 mM diazepam, the classical benzodiazepine agonist reported to increase the affinity of the GABAA receptor to GABA (16, 27). Figure 3 shows that diazepam did not change the frequency (TTX + DNQX/urethane: 0.6 ± 0.2 Hz; TTX + DNQX + urethane + diazepam: 0.7 ± 0.2 Hz; Fig. 3A) and the mean amplitude (TTX + DNQX + urethane: 27.6 ± 2.7 pA; TTX + DNQX + urethane + diazepam: 31.2 ± 3.8 pA; Fig. 3B) of the events, but it increased the mIPSC half-width (TTX + DNQX + urethane: 5.9 ± 1.6 ms; TTX + DNQX + urethane + diazepam: 13.5 ± 1.2 ms; Fig. 3C). The lack of effect of diazepam on the mean amplitude shows that the GABA_A receptors are saturated by synaptic release of GABA in the NTS slice. The increase of the mIPSCs mean half-width by diazepam is in accordance with an already reported benzodiazepine saturation-independent effect on the GABA channel deactivation (16, 20).

Urethane does not affect the ST ePSCs. Although urethane did not affect the glutamatergic spontaneous transmission, we also investigated its effect on glutamate-ePSCs after electrical stimulation of the ST. In the presence of bicuculline and at a holding potential of −70 mV, stimulation of the ST produced inward fast-decaying evoked excitatory postsynaptic currents (eEPSCs) that were abolished by DNQX (data not shown), indicating that non-NMDA subtypes of glutamate receptors (AMPA/kainate) were activated. In accordance with the lack of effect of 20 mM urethane on the non-NMDA sEPSCs, Fig. 4A shows that this anesthetic also had no effect either on the non-NMDA eEPSC amplitude (control: 204.4 ± 35.5 pA before and 196.6 ± 31.2 after 20 mM urethane, n = 6) or on its kinetics. We tested the effect of 60 mM urethane on the non-NMDA eEPSC, and we also found no effect on the amplitude of the events (control: 176 ± 33.1 pA before and 156.6 ± 31.2 after 60 mM urethane, n = 6).

The effect of urethane was also tested on the NMDA eEPSC. For measuring pure responses to NMDA receptor activation, we blocked the fast currents generated by activation of non-NMDA receptors with DNQX and depolarized the neuron to a positive potential (40 mV) to relieve the magnesium blockade of the NMDA receptors. Figure 4B shows that urethane did not have significant effect either on the peak amplitude (44.2 ± 11.5 pA before and 37.6 ± 10.6 pA after urethane, n = 6) or on the activation and inactivation kinetics of the NMDA eEPSC.

![Fig. 2. Summary of the effects of urethane (20 mM) on the frequency (A), amplitude (B), and half-width (C) of glutamatergic spontaneous excitatory postsynaptic currents (sEPSCs) in NTS neurons (n = 6). Bic, bicuculline.](image-url)

![Fig. 3. Summary of the effects of urethane (20 mM) and diazepam (1 mM) on the frequency (A), amplitude (B), and half-width (C) of GABAergic miniature sIPSCs in NTS neurons (n = 8). DZ, diazepam.](image-url)
Urethane does not affect the excitability and passive properties of the NTS neurons. Action potentials (Fig. 5, A and B) were elicited by injecting depolarizing current in the NTS neurons, and urethane did not change either the number of action potentials (Fig. 5C) or the threshold of the first action potential that follows the current injection (Table 1). After 5 min of addition of urethane in the bath perfusion, there was no change in resting membrane potential, the input resistance, or the membrane time constant of the NTS neurons (Table 1).

DISCUSSION

Urethane induces deep narcosis and is widely used in animal experimentation, mainly because of its long-lasting action (18, 19). However, several studies show that urethane affects the neurotransmission in the CNS, although the mechanisms are not yet completely understood (4, 7, 9, 11, 12, 17).

Urethane may affect the neural processing of several physiological functions. Cross and Silver (9) have shown that the response of the rabbit hypothalamic neurons to a large variety of stimuli (thermal, painful, auditory stimuli, as well as to hypoxia and hypercapnia) is affected by urethane. Angel and Gratton (4) demonstrated that an increase in the depth of anesthesia upon administration of urethane in rats produced a dose-related change in the magnitude and latency of the evoked response by stimulation of the forepaw. The autonomic responses also change after urethane administration. It has been shown that urethane depresses the baroreceptor reflex (12) and reduces the pressor and bradycardic responses induced by norepinephrine microinjection in the cingulate cortex of rats.

Table 1. Passive membrane properties of NTS neurons before and after urethane addition to the bath perfusion

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Urethane (20 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP, mV</td>
<td>−64±1.19</td>
<td>−63.51±3.87</td>
</tr>
<tr>
<td>Threshold, mV</td>
<td>−45.12±2.09</td>
<td>−49.05±3.39</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>701.6±98.3</td>
<td>755.7±159.3</td>
</tr>
<tr>
<td>Membrane time constant, ms</td>
<td>34.15±5.86</td>
<td>43.69±8.50</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 cells. NTS, nucleus of the solitary tract; RMP, resting membrane potential. For details, see Fig. 5.
A previous study from our laboratory (17) showed that microinjection of L-glutamate in the NTS of awake rats produced a large increase in MAP, whereas in the same rat under urethane anesthesia, the microinjection of the same dose of L-glutamate produced a fall in the MAP. Taken together, these data clearly show that urethane anesthesia may affect the neural processing in several areas of the CNS, including the NTS.

The mechanisms of action of urethane are unknown, but it has been proposed that some types of general anesthetics (volatile and gaseous anesthetics and nonimmobilizer drugs) increase the GABAergic inhibitory currents and/or inhibit the glutamatergic responses (10). Although there is a large variety of neurotransmitters and neuromodulators within the NTS (15), L-glutamate and GABA are considered the major neurotransmitter systems at this level. It is well accepted that glutamate is the neurotransmitter released by the first-order visceral afferent fibers on neurons within the NTS, and GABA would be only released by activation of GABAergic interneurons (2).

There is evidence indicating that GABA exerts a powerful modulatory role in the NTS on the regulation of respiration, arterial blood pressure, and heart rate (25, 30). Our results showed that urethane reduces the frequency of spontaneous GABAergic synaptic currents and does not affect the glutamatergic neurotransmission (spontaneous or evoked events), suggesting that most of the GABAergic mechanisms in the NTS may be affected in experiments performed under this type of anesthesia.

Some studies have reported different actions of urethane on GABAergic neurotransmission (8, 13, 21). The depolarization of the superior cervical ganglion of the rat was reduced in the presence of bicuculline, and urethane was effective in reversing the effect of bicuculline, suggesting that this anesthetic could increase the action of GABA (8). There is evidence that urethane enhances the action of GABA in GABA_\text{A} receptors expressed in \textit{Xenopus} oocytes, possibly by increasing the affinity of the GABA_\text{A} receptors to GABA, resulting in larger GABA_\text{A} receptor currents (13). However, this effect will occur only if the GABA concentration released at the synaptic cleft does not saturate the receptor; otherwise, urethane will not affect the amplitude of the inhibitory current.

In the present study, urethane did not change the mIPSC amplitude, suggesting that 1) urethane has no effect on the GABAergic postsynaptic receptors in NTS neurons or 2) the postsynaptic receptors are saturated by the concentration of GABA released, and urethane cannot affect the amplitude of the inhibitory events. To verify this hypothesis, we analyzed the effect of diazepam, a benzodiazepine agonist that increases the affinity of the GABA_\text{A} receptor to GABA (16, 27). The data showed that diazepam did not change the mIPSCs amplitude in the NTS neurons, supporting the concept that GABA_\text{A} postsynaptic receptors in our experimental condition are saturated by GABA, masking any possible effect of urethane on the mIPSC amplitude.

We did not observe a decrease in the amplitude of the glutamatergic spontaneous and evoked (both NMDA and non-NMDA) events, although it has been reported that urethane decreased the AMPA- and NMDA-induced currents (13). However, the effect of urethane on receptor ion channels observed by Hara and Harris (13) presented an EC_{50} higher than the concentrations of urethane used in our study (20 mM), which is closer to its plasma concentration during surgical anesthesia (18, 19, 29). This result may imply that the urethane effect on glutamatergic currents would be observed at a higher concentration. Nevertheless, in the present study, we verified that a threefold higher concentration of urethane (60 mM) also did not change the mean amplitude of sPSCs or the evoked non-NMDA EPSC in NTS slices. Therefore, even if we assume that urethane has postsynaptic effects on ionotropic GABA and glutamate receptors, these effects are not changing the amplitude and waveform of either IPSCs or EPSCs in the NTS.

Our results suggest that urethane inhibits GABA release by diminishing the probability of firing GABAergic interneurons in the NTS without decreasing the release probability of GABAergic synaptic vesicles. This suggestion is supported by the following observations: 1) urethane does not affect the mean amplitude of IPSCs, and 2) it does not affect the frequency of TTX-insensitive mIPSCs. However, considering that urethane had no effect on the passive and firing properties of the NTS neurons, it might be possible that urethane is also acting on a small subset of neurons in the NTS, which, by chance, were not recorded in our experiments. The present results are unexpected because they show that, in the NTS, urethane does not present the usual effects of other general anesthetics on neurotransmitter receptors in CNS (10).

It has also been reported that urethane affects GABAergic transmission in other brain areas, such as the rostral ventrolateral medulla (RVLM), an important brain stem region involved in the generation of the sympathetic activity to the cardiovascular system. In conscious rats, microinjection of GABA in RVLM induced a small decrease in blood pressure. However, in the same animals, the fall in blood pressure was greater after urethane anesthesia (15). Either GABA_A receptor antagonists (bicuculline) or GABA_B receptor antagonists (2-hydroxyysaclofen or CGP-35348) microinjected in the RVLM produced a pressor response in urethane-anaesthetized rats (1, 5), suggesting that, at least in the RVLM, urethane is not producing inhibition of the tonic activity of GABAergic neurons projecting to this region. However, no experiments with the use of patch-clamp recordings were performed to explore the effect of urethane on RVLM neurons.

We conclude that urethane does not affect postsynaptic excitatory and inhibitory neurotransmission in the NTS, but it reduces the GABAergic drive tonus by diminishing the frequency of action potential-dependent GABAergic synaptic currents. These results show that urethane, in concentrations similar to those used in the anesthetized whole animals, changes the dynamics of the synaptic circuitry in the brain stem, which may deeply affect the processing of the cardiovascular and other reflexes at the NTS level. Therefore, the effect of urethane in in vivo preparations involving CNS studies is a very important variable that must be taken into consideration when interpreting the results obtained under this experimental condition.

**ACKNOWLEDGMENTS**

We thank Adriana A. B. Murashima for technical assistance.

**GRANTS**

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo Grants 03/05016–3 and 04/03285–7 and Conselho Nacional de Desenvolvimento Científico e Tecnológico Grant 472704/04–4.
REFERENCES

1. Amano M, Kubo T. Involvement of both GABA_A and GABA_B receptors in tonic inhibitory control of blood pressure at the rostral ventrolateral medulla of the rat. Naunyn Schmiedebergs Arch Pharmacol 348: 146–153, 1993.


