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Increase in plasma ACTH induced by urethane is not a consequence of hyperosmolality

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Abstract

Although anesthetic doses of urethane increase plasma levels of ACTH, the exact mechanism through which this occurs is unclear. We theorized that these increases might be a consequence of an increased systemic osmolality owing to the large doses of urethane usually employed. To evaluate this possibility, we measured plasma osmolality and ACTH in a total of six rats after graded infusions of urethane (N=3 rats) or equimolar amounts of mannitol (N = 3 rats). Rats received infusions at 15 min intervals up to a cumulative dose equivalent to an anesthetic dose for urethane (1.4 g/kg). Blood samples (0.35 mL) were withdrawn at baseline and 10 min after each infusion. Urethane and mannitol produced significant and equivalent increases in plasma osmolality. However, only urethane evoked increases in plasma ACTH which were maximal (252 ± 55 pg/ml from a baseline of 27 ± 7 pg/ml) after a cumulative dose of 1 g/kg. Thus, increases in plasma ACTH seen after anesthetic doses of urethane are unlikely to be a consequence of its effect on plasma osmolality.

Keywords

urethane; osmolality; HPA axis; ACTH

INTRODUCTION

In 1885 Oswald Schmiedberg, a professor of pharmacology at the University of Strasbourg, first described the anesthetic properties of urethane (ethyl carbamate) (19). Although its mechanism of actions is still not known, its low cost, ease of administration, long duration of action and muscle relaxant properties make it widely used anesthetic in animal research even today (18,22,24). In contrast to barbiturates, urethane activates the hypothalamic-pituitary-adrenal (HPA) axis (6,30); an effect that is independent of its transformation into its major metabolite hydroxyurethane (31). Activation of HPA has also been described for ethyl ether (10) and ketamine (8,16). Urethane's activation of the HPA axis occurs at doses that are much lower than those required for anesthesia: Urethane evokes maximal increases in circulating

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corticosterone levels at a dose of 1 g/kg (31), while 1.0-1.5 g/kg is generally required to achieve stable levels of surgical anesthesia (19). Therefore, even low doses of urethane (0.4 g/kg) – commonly used to potentiate effects of chloralose – are sufficient to evoke significant increase of corticosteroid release.

Activation of the HPA axis induced by urethane likely has a central origin at the level of the hypothalamic-hypophyseal complex (11). An administration of this anesthetic dramatically increases fos-like immunoreactivity in the parvocellular subdivision of the hypothalamic paraventricular nucleus (PVN; 25).

Because urethane requires doses above 1 g per kg of body weight for the induction of anesthesia, solutions of urethane injected may have concentration as high as 15 M; as such, they have the potential to significantly increase plasma osmolality (27). Induction of a hyperosmotic state by urethane anesthesia may account for its ability to induce strong fos-like immunoreactivity in magnocellular subdivision of the PVN, which contains both vasopressin- and oxytocin-containing neurons (25). Consequently, anesthesia with this agent may constitute a significant problem in studies of where levels of these hormones or their actions are assessed (22).

In addition to regulating arginine vasopressin (AVP) secretion, plasma osmolality affects ACTH secretion. In both horses and rats, infusions of hyperosmotic solutions of saline increase plasma ACTH secretion (13,14,26). Similar effects have been demonstrated in humans (7). The converse of this is also true: hyposmolality blunted increases in ACTH evoked by lipopolysaccharide and the anesthetic ether (4,5). While not completely understood, the mechanism by which osmolality affects ACTH secretion involves synergistic action between AVP and corticotropin releasing factor (CRH) (33)

In this study we aimed to determine whether the effect of urethane on plasma ACTH might be a function of its ability to increase plasma osmolality. Therefore, we assessed the effect of urethane and mannitol on plasma ACTH in parallel experiments where these agents were administered at cumulative doses that produced comparable effects on plasma osmolality.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (300-320 g) were individually housed under standard controlled conditions (lights on 0700-1900, 23-25°C) with free access to food and water. All procedures described were approved by the Institutional Animal Care and Use Committee and followed NIH guidelines.

Surgical Procedures

All rats (n=6) were anesthetized with ketamine/xylazine (80 mg/kg ketamine; 12 mg/kg xylazine, ip) and instrumented with catheters in a femoral artery for blood sampling and drug administration. A small incision was made in the inguinal region and the femoral artery was exposed and separated from surrounding tissues. Tygon tubing (0.02 in.ID) tipped with a small diameter (0.015 in ID) Teflon tubing segment was filled with saline and inserted, Teflon segment first, approximately 5 cm into the artery. The catheter was routed subcutaneously and exteriorized in the interscapular region. All incisions were closed with small sutures. The catheter was stabilized at the nape of the neck with a small leather harness. Our choice of intra-arterial administration excluded the possibility of activation of the HPA secondary to abdominal organ damage or hypotension – effects which have been shown to occur with intraperitoneal but not intra-arterial administration (19,32). In addition, as an arterial catheter was needed for blood withdrawal, we used an experimental design employing a single catheter

thereby reducing surgical burden. Rats were allowed to recover at least for 5 days before experiments.

All animals remained in good health throughout the course of surgical procedures and experimental protocols as assessed by appearance, behavior and maintenance of body weight.

Experimental Protocol

All experiments were performed between 10:00 am and 2:00 pm to avoid the effect of circadian variability on plasma levels of ACTH. Animals were brought to the laboratory and allowed to accommodate for at least 2 hours. Each animal was randomly assigned to receive either urethane (URE) or mannitol (MAN). Urethane and mannitol were slowly injected intraarterially over a 1 min interval as equimolar solutions (0.75 M: urethane – 0.0675 g/ml, mannitol – 0.138 g/ml). Blood samples (0.35 ml) were withdrawn at 0, 15, 30, and 45 min followed in each case by injections of either urethane or mannitol at cumulative doses of 0.2, 0.6, 1.0, 1.4 g/kg and 0.4, 1.2, 2.0, 2.8 g/kg, respectively (volumes 1 ml/300 g of b.w. at 0 min and 2 ml/300 g of b.w. at 15, 30, and 45 min).

Blood sampling and analysis

Blood samples were collected in syringes containing 60 μ l of a solution of 10 mg/ml EDTA and 50% aprotinin (Sigma, USA) in saline. Blood was immediately centrifuged at 4°C and divided into two aliquots. Osmolality was immediately measured in 10 μ l plasma samples from first aliquot using a Vapro 5520 osmometer (Wescor Inc, Logan, UT, USA). Individual measurements were repeated in triplicate and the results were averaged.

Another aliquot of the plasma was stored at -80°C until analysis for ACTH content by radioimmunoassay (34). Briefly, rabbit polyclonal ACTH antiserum was purchased from IgG Corp. (Nashville, TN), ¹²⁵I-ACTH - from Diasorin (Stillwater, MN). The antigen-antibody complex was sedimented with goat anti-rabbit serum (Calbiochem, San Diego, CA). Samples were used in duplicates after appropriate dilution in order to be in the linear part of the calibration curve (1-10 pg ACTH/tube).

Statistical analysis

The results are presented as the mean \pm SEM. Results were compared using a one way ANOVA with repeated measures followed by a Fisher's LSD post hoc test. A value of $P < 0.05$ was considered to indicate a significant difference in all comparisons.

RESULTS

There were no statistically significant differences between groups (URE vs MAN) in baseline levels of ACTH (27 ± 7 vs 22 ± 15 pg/ml, $F(1,4)=0.12$, $p=0.74$) and osmolality (251 ± 13 vs 268 ± 8 mOsm, $F(1,4)=1.21$, $p=0.33$). Injection of urethane resulted in a clear increase in plasma osmolality even at the lowest dose (Figure). Equimolar injection of mannitol produced changes in plasma osmolality that were not significantly different from those evoked by urethane ($F(1,4)=0.33$, $p=0.60$ for group comparison and $F(4,16)=0.96$, $p=0.45$ for group-dose interaction).

Administration of urethane clearly increased plasma ACTH, an effect that was significantly different from that of mannitol ($F(1,4)=13.18$, $p=0.02$ for group comparison). Plasma levels of ACTH were significantly increased at all time points after administration of a total of 0.6 g/kg urethane whereas mannitol failed to do so even after the highest cumulative dose.

DISCUSSION

Urethane is known for very slow elimination: after single intraperitoneal administration the plasma concentration of urethane decreases with time from 16 mM at 1 h to 12 mM at 6 hours (23). Therefore, in this study we used cumulative dose after multiple injections which were performed within 1 hour.

In our experiments activation of HPA axis by urethane was clearly evident in all rats. Increases in plasma ACTH were essentially maximal after 0.6 g/kg. This dose is not sufficient to induce surgical anesthesia by itself, but it is similar to doses used in combination with alpha-chloralose (range of 0.3 – 0.5 g/kg of urethane). These observations are in agreement with reports on corticosterone release suggesting that maximal responses are evoked by relatively low doses of urethane, in the range of 0.6-0.7 g/kg (31).

Our data support our original supposition that administration of anesthetic doses of urethane induces hyperosmolality of blood plasma. In fact, the lowest dose of urethane (0.2 g/kg) significantly increased osmolality in our experiments. The increase of plasma osmolality (10-15 mOsm after 1.4 g/kg) is consistent with plasma concentrations observed after same dose of urethane was administered intraperitoneally (16 mM, 1 hour after injection; 23). Mannitol did not activate the HPA at any dose, including those which significantly increased blood plasma osmolality. Therefore, in contrary to other studies, in which hyperosmolality was induced by sodium chloride (14,26), osmolality by itself is not sufficient to induce increases of pACTH. We conclude that hyperosmolality induced by urethane is not the cause of HPA activation.

The mechanism responsible for activation of brain circuitry relevant to the observed effect of urethane on the HPA axis is unknown. Modulation of cardiovascular control by urethane was hypothesized to be mediated by alpha2-adrenoreceptors (2,28). However, effects of urethane on adrenoreceptors were never demonstrated directly and the relevance of this type of receptors to activation of HPA induced by urethane is unknown.

More recently, Hara&Harris (12) described effects of urethane on neurotransmitter-gated ion channels expressed in *Xenopus* oocytes. The *in vitro* concentrations of urethane used in this study are in the range of plasma concentrations of urethane injected to reach surgical anesthesia (15). Either enhancing inhibitory tone or inhibiting responses of excitatory receptors has a potential to activate the HPA axis. We have demonstrated that inhibition of neuronal activity in the preoptic area results in powerful release of ACTH (34). However, it is impossible to confirm that the 10-20% changes of transmembrane currents found *in vitro* are ethiological to induction of physiological responses of the whole animal.

Regardless of the mechanism involved in these effects of urethane, the results of our study renew the controversy regarding the suitability of urethane as an anesthetic agent in experimental studies (19,20,21). The ability of urethane to activate both parvocellular and magnocellular subdivisions of the PVN (25) and the central noradrenergic system (28); to elicit hyperglycemia (1), and the centrally-mediated peripheral release of epinephrine and norepinephrine (2,9,29) suggest that a wide range of specific consequences may accompany urethane anesthesia. These effects are physiologically significant: plasma levels of both epinephrine and norepinephrine after 1.5 g/kg of urethane (9) are comparable with those induced by emotional stress (17).

Even though the use of urethane for experimental anesthesia can be justified in some studies (3), its use should be carefully considered.

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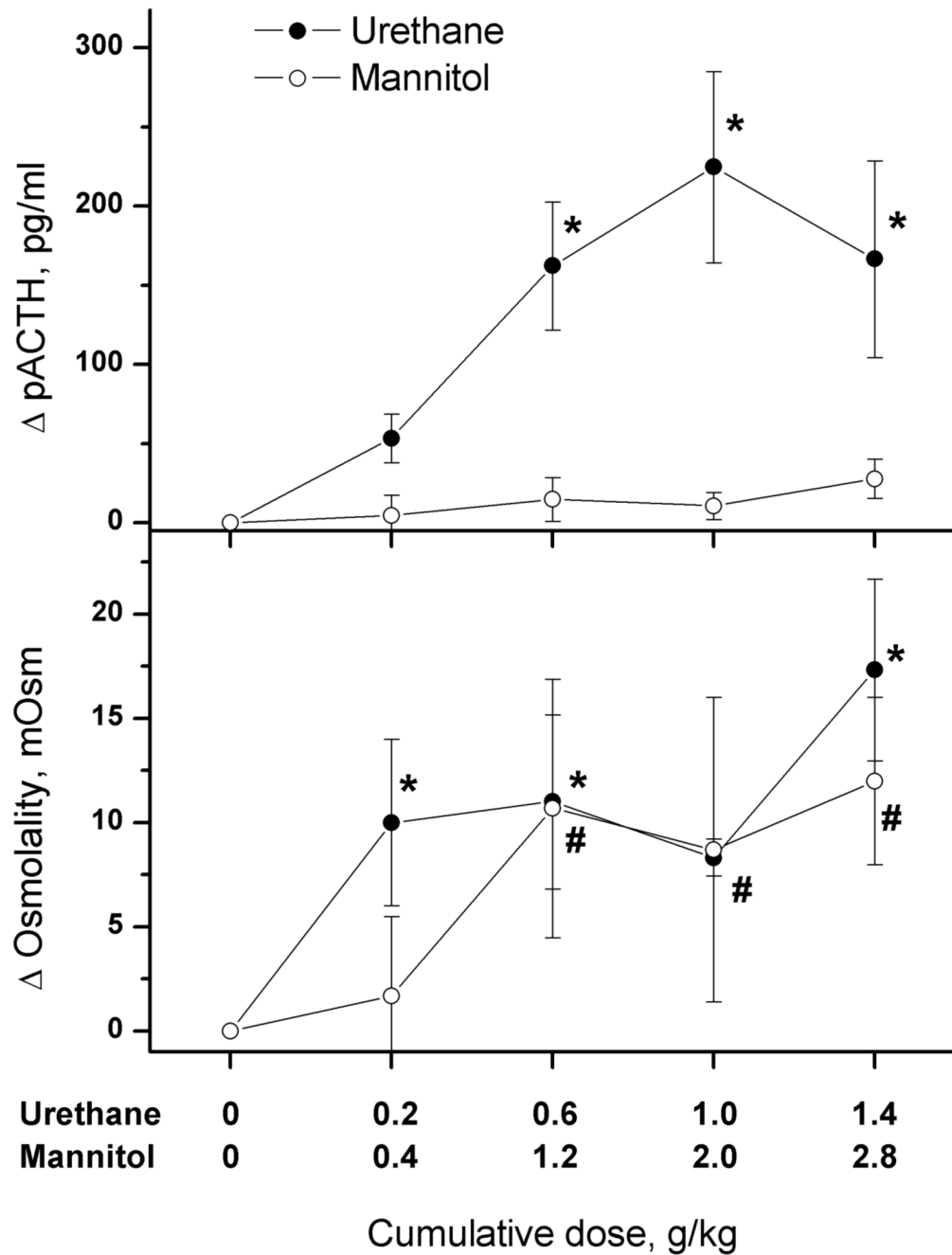


Figure. Changes of plasma ACTH (A) and osmolality (B) induced by intraarterial injections of urethane (closed circles) or mannitol (open circles). * - significant changes from baseline for urethane ($p < 0.05$); # - significant changes from baseline for mannitol ($p < 0.05$).