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Reviews

Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 1: General considerations

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Summary. The suitability of urethane anesthesia for physiopharmacological investigations is reviewed. Total dose administered and route of administration are recognized as factors having a great influence on both resting parameters and biological responses to drugs. A peculiar characteristic of urethane is represented by its ability to induce a surgical plane of anesthesia without affecting neurotransmission in various subcortical areas and the peripheral nervous system. This makes urethane a suitable general anesthetic for studying neural function in both central and peripheral nervous systems and accounts for the preservation of a number of reflex responses in urethane-anesthetized animals.
Key words. Urethane; anesthesia; physiology; pharmacology; in vivo experiments; reflexes.

Introduction

It is well known that anesthetics have profound effects upon various physiological variables as well as on responses of various test systems to pharmacological stimulation. However, in many experiments, anesthesia is necessary both for ethical reasons and for practical purposes.

The knowledge of potential interference of the anesthetic used with parameters under study is of paramount importance for assessing the physiological relevance of data obtained in anesthetized animals. A reasonable estimate of the potential interference of anesthesia requires a basic knowledge of the effects produced by the dose of the anesthetic given, by the particular route of administration used on the parameter(s) examined in the animal species under study. Therefore a critical review of the suitability of a certain anesthetic for physiopharmacological studies in various systems might be helpful for selecting the proper anesthetic for performing a certain type of experiment.

Our aim is to review some evidence concerning the suitability (or unsuitability) of urethane anesthesia for physiopharmacological experimentation in various systems. Rather than trying to make a list of the complete literature available on this topic we shall focus our attention on those studies in which the suitability of urethane anesthesia has been specifically checked by the authors.

Urethane (ethyl carbamate) was introduced as an anesthetic by Schmiedeberg in 1885. He stated that this substance induces profound narcosis with little change in respiration or circulation. Nowadays urethane is widely used for animal experimentation, mainly because of its long duration of action and skeletal muscle relaxant properties. In particular, urethane is recommended for acute experiments involving study of reflex responses[12,61,63,65].

While these advantages confer popularity on the use of urethane as an anesthetic a number of studies indicate that this substance interferes with various physiological parameters. Also, the pharmacological responses of various test systems are affected, either directly or indirectly, by urethane anesthesia.

In the first part of this review we shall try to pinpoint certain principles which should be examined carefully before deciding in favor of or against the suitability of a given anesthetic under given experimental conditions. In the following sections of this review (part 2: Cardiovascular system; part 3: Other systems and Conclusions) we shall examine the suitability of urethane anesthesia in specific areas and biological systems.

Before examining the potential effects of urethane anesthesia on various systems some consideration is needed concerning the importance of factors such as dose and route of administration. It is our recommendation that each 'life scientist' should check carefully the suitability of this (and other) anesthetics when extrapolation from published data is made for factors such as total dose administered, route of administration and animal species.

Urethane anesthesia: relevance of total dose administered and plasma concentrations on the response under study

It is reasonable to assume that the influence of a given anesthetic on a given test system is, to a certain extent, dose-related. Therefore the minimal dose required to produce a suitable level of surgical anesthesia should be used. This recommendation stems from the assumption that

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deepening of anesthesia with increasing dose of the anesthetic is likely to produce a progressive depression of either excitatory or inhibitory mechanisms controlling the responses of the target organ. These uncontrolled variables might produce changes of unpredictable magnitude in the resting function of the target organ and/or the quality and magnitude of the response of the test system to pharmacological stimulation. In this and the following sections of this review the term 'suitable level of surgical anesthesia' refers to a condition which allows the performance of the surgical procedures required for a given type of experiment without producing pain or discomfort to the animal.

Intraperitoneal (i.p.) urethane is commonly administered to induce surgical anesthesia in rats at doses of 1.2–1.5 g/kg but lower doses (0.8–1.1 g/kg) are sufficient for induction of a suitable level of surgical anesthesia[35,50,51]. For instance Lincoln et al.[45] reported that 1.1 g/kg of i.p. urethane induces a long-lasting surgical anesthesia which is suitable for stereotaxic manipulation and neurosurgery for at least 8 h.

These factors should be taken into account when critically examining the literature concerning side effects and contraindications of a given anesthetic for animal experimentation. In figure 1 is shown a clearcut example of the influence of small increases in the dose of urethane on the response under study. In fact a 15–30% increase in the dose of urethane depressed markedly the amplitude of the bladder contraction produced by the excitatory cutaneo-vesical reflex in rats. It should be noted that: a) this reflex is organized at spinal level[56,57] and b) urethane, in concentrations slightly above the minimal ones required for surgical anesthesia (10–15 mM)[18,42], depresses markedly the dorsal root-evoked ventral-root potentials in the spinal cord[32,33]. These observations emphasize the need for using, in each experimental condition, the minimal dose of urethane required to perform surgery without producing discomfort or pain to the animal.

In the light of the above an analysis of the literature concerning the effects of urethane anesthesia on various functions and, consequently, an evaluation of its suitability for physiopharmacological experiments, needs careful evaluation in relation to the dose of anesthetic used.

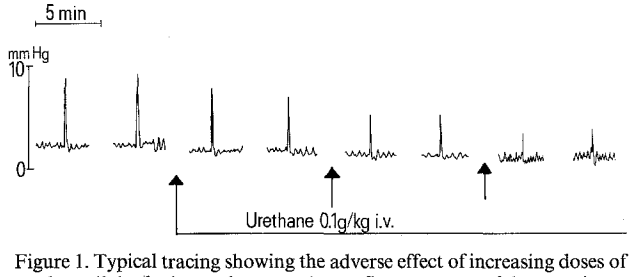

Figure 1. Typical tracing showing the adverse effect of increasing doses of urethane (0.1 g/kg i.v. at the arrows) on reflex responses of the rat urinary bladder obtained following pinching of the perineal skin as described by Sato et al.[56,57] (cutaneo-vesical excitatory reflex). Initial anesthesia was induced by s.c. urethane 1.2 g/kg. The bladder was filled with an amount of saline (0.2 ml) insufficient to elicit the vesico-vesical micturition reflex. Perineal pinching induced reproducible bladder contractions at 10-15-min intervals. This tracing is representative of 5 observations. In some experiments the reflex was abolished at urethane dose (total dose administered) of 1.4–1.5 g/kg.

Boyland and Rhoden[18] reported that s.c. urethane (1 g/kg) induces surgical anesthesia only in a fraction (not given) of preparations. Threshold plasma concentrations for induction of anesthesia range between 60 and 80 mg/100 ml. However, a plasma concentration of at least 80 mg/100 ml (about 10 mM) was required to obtain surgical anesthesia. This observation was confirmed by subsequent studies reporting that urethane plasma concentrations equal to or higher than 10 mM produce surgical anesthesia[13,14,42,66].

In figure 2 we present data concerning the time course for the development of surgical anesthesia in male albino rats of the Wistar Morini strain receiving s.c. urethane (1.0–1.2 g/kg), i.e. the anesthetic regimen currently used in our laboratory. It should be noted that s.c. urethane (1.2 g/kg) produces a long lasting (up to 6 h) surgical anesthesia characterized by skeletal muscle relaxation and preservation of certain reflex responses (corneal reflex). The lower dose tested (1.0 g/kg) induces a suitable level of surgical anesthesia in a lower percentage of rats, and anesthesia developed more slowly (fig. 2).

Urethane pharmacokinetics

In rats receiving s.c. urethane (1.0 g/kg) similar levels of urethane were observed in blood and organs, including brain[18]. Urethane is largely (and slowly) metabolized (to ethanol and carbamic acid) before being excreted[18,19,62,65]. Therefore it might be anticipated that factor(s) which influence the liver metabolizing capacity, such as age and sex, could induce variations in: a) the minimal dose of

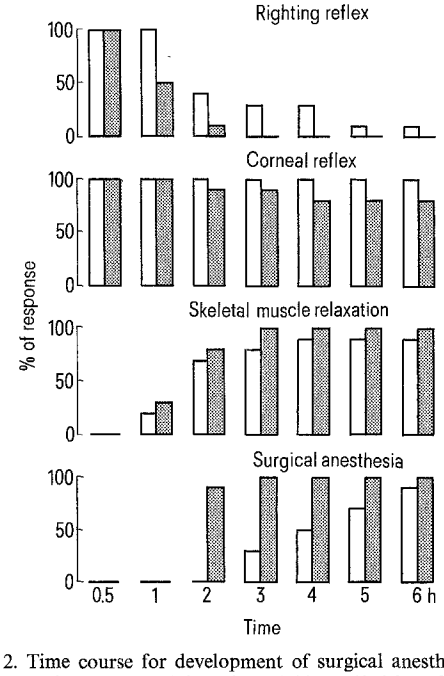

Figure 2. Time course for development of surgical anesthesia in male albino rats of Wistar Morini strain weighing 340–360 g following s.c. administration of urethane 1.0–1.2 g/kg s.c. Note the maintenance of corneal reflex and presence of skeletal muscle relaxation during urethane induced surgical anesthesia. Skeletal muscle relaxation was evaluated by holding a fold of skin on the ventral surface of the thorax and looking for the presence of nuchal relaxation. Surgical anesthesia was judged by the absence of response following incision of the abdominal skin.

urethane required to induce surgical anesthesia; b) duration and depth of anesthesia; c) the influence of urethane on resting functions, and d) the influence of urethane on response to drugs.

Urethane anesthesia and its influence on activity of the sympathetic nervous system

One of the major drawbacks of the use of urethane anesthesia derives from the notion that urethane activates the sympathetic outflow from the central nervous system (CNS) to peripheral organs[8,53]. It has been known for a long time that urethane-anesthetized animals have abnormally high blood glucose levels[10,21,27,60]. Reinert[53] showed that: a) the urethane-induced hyperglycemia is prevented by reserpine pretreatment; b) hypothalamic noradrenaline levels and catecholamine content in the adrenal medulla are reduced after i.p. urethane (1.25 g/kg) and, c) i.v. urethane (0.2–0.8 g/kg) increases discharge of the preganglionic sympathetic filaments in fluothane anesthetized cats[53].

Reinert[53] concluded that urethane-induced hyperglycemia may be a consequence of hypothalamic or general limbic activation resulting in increased sympathetic discharge, which, in turn, leads to enhanced catecholamine secretion from the adrenal medulla[9,40,53,55]. These findings were confirmed by subsequent investigations which, however were performed almost exclusively in rats receiving a rather high dose (usually 1.5 g/kg) of i.p. urethane[8,36,28,29,64].

Taken together these findings provide evidence indicating that, in various species (and particularly in rats) i.p. urethane at doses equal to or higher than 1.2 g/kg produces an activation of sympathetic structures at CNS level leading to increased catecholamine secretion from the adrenal medulla, and hyperglycemia.

In the meantime there is not, in our opinion, sufficient information to conclude that these adverse effects are necessarily observed as a consequence of urethane anesthesia. In particular, no data are available on plasma levels of adrenaline in urethane-anesthetized animals after the s.c. or i.v. administration of the minimal dose required to produce surgical anesthesia. Therefore, while i.p. urethane should be contraindicated for experiments involving physiopharmacological experiments on the sympathetic nervous system, the question of its suitability by other routes remains open. The relevance of choosing the proper route of administration to avoid certain side effects of urethane will be examined in the following section.

Relevance of the route of administration to minimize some of the adverse effects of urethane anesthesia

Urethane is often administered by the i.p. route mainly because of the short time required to attain a surgical level of anesthesia. Various studies suggest that i.p. urethane (1.3–1.5 g/kg) has profound endocrine and metabolic effects which can interfere markedly, both qualitatively and quantitatively, with the results of physiopharmacological studies. In the meantime some observations suggest that certain adverse effects of urethane anesthesia are much less intense or even absent when this substance is administered by routes other than the i.p. one[15,31,61,67]. Van der Meer et al.[67] reported that, in rats, i.p. urethane (1 g/kg) produces a marked increase in hematocrit along with a fall in plasma protein and superficial damage of intraabdominal organs (necrosis of the liver, spleen, pancreas and mesenterium).

Hemoconcentration was attributable to a massive leakage of plasma into the peritoneal cavity, leading to hypotension[67]. These side effects were not observed following administration of the same dose of urethane by the oral or i.v. route and were markedly reduced in animals receiving s.c. urethane[67]. It was concluded that urethane-induced necrosis of the intraabdominal organs was responsible for systemic side effects observed following its i.p. administration[67].

In the same study it was also reported that i.p. but not intrarterial (i.a.) urethane induces hyperglycemia. As outlined in the preceding section this adverse effect of urethane appears to be related to an increased activity of the sympathetic nervous system at hypothalamic-limbic level[8,27,53]. The observation that the hyperglycemia was not observed followed i.a. urethane suggests that stimuli arising from tissues damaged by the i.p. urethane were responsible for the sympathetic activation at CNS level. This hypothesis is supported by findings obtained in our laboratory indicating that blood glucose levels are slightly affected in rats receiving s.c. urethane (1.0–1.2 g/kg) during an observation period of 4 h[31]. The hypothesis that activation of sympathetic structures in the CNS (leading to an increased sympathetic outflow to the periphery) following i.p. urethane depends upon an irritation of sensory structures in the peritoneum rather than on an effect of urethane anesthesia 'per se' awaits testing. Considering that suitable levels of surgical anesthesia could be obtained by other routes of administration, i.p. urethane should be avoided unless there are specific reasons for its use.

Urethane and GABAergic neurotransmission

In recent years much attention has been given to the potential effect of anesthetics on GABAergic neurotransmission. These studies led to the conclusion that certain anesthetics (notably barbiturates) enhance GABAergic neurotransmission[11,20,26,54] at concentrations similar to those found in plasma or brain of anesthetized animals. The knowledge of a potential interference of anesthetics with GABAergic neurotransmission is of paramount importance in physiological studies and also for evaluating, during acute 'in vivo' experiments, the effect of substances having a known or suspected action on GABAergic neurotransmission. The recent discovery that GABA is a neurotransmitter in the peripheral nervous system also[17,37,38] suggests that the anesthetic used could influence certain physiological parameters and/or pharmacological responses in peripheral tissues through an action(s) on GABAergic neurotransmission.

Bowery and Dray[16] reported that urethane reverted the antagonistic effect of bicuculline on GABA-induced depolarizations in the isolated rat superior cervical ganglion. However other reports indicate that urethane does not interfere significantly with GABAergic neurotransmission. Scholfield[59] showed that urethane (10–50 mM)

was the least potent of a series of anesthetics in producing enhancement of the inhibitory postsynaptic potential in guinea pig olfactory cortex, a type of response which seems to involve a GABAergic mechanism[58,59].

Evans and Smith[32,33] reported that the depressant action of urethane on neurotransmission in the spinal cord was unaffected by a concentration of picrotoxin which reduces markedly the depressant effect of amylobarbitone. Minchin[49] reported that urethane, in anesthetic-like concentrations (0.5–30 mM), does not modify the uptake of GABA nor its spontaneous or K^+-stimulated release from rat cortical slices. In a later study similar findings were obtained on rat thalamic preparations[39]. Moroni et al.[50] reported that the release of endogenous GABA from the rat cerebral cortex of urethane anesthetized rats (0.89 g/kg i.p.) does not differ from that of unanesthetized animals. Lalley[41] studied the effect of various anesthetics on certain pressor or depressor cardiovascular reflexes. Unlike barbiturates, urethane did not influence the central GABAergic mechanism involved in the control of cardiovascular functions, although it reduced the amplitude of the reflex response with increasing depth of anesthesia.

In conclusion these findings indicate that, compared with other anesthetics, urethane produces only minimal or no enhancement of GABAergic neurotransmission at the level of the central and, possibly, of the peripheral nervous system. Therefore urethane anesthesia may be considered suitable (or, in any case, better suited than that produced by other anesthetics) for physiopharmacological investigations concerning GABAergic neurotransmission and its modulation by drugs.

Effect of urethane anesthesia on neuronal function in various areas of the central nervous system

One of the distinctive features of urethane anesthesia is represented by its slight (as compared to other anesthetics) depressant effect on reflex responses. This characteristic has a functional counterpart in the observation that the activity of various subcortical structures of the CNS is barely affected by urethane.

Evans and Smith[32,33] reported that urethane reduces the ventral root potential evoked by electrical stimulation of the corresponding dorsal root in the frog spinal cord. The ED50 for this effect was about 50 mM, while anesthetic concentrations (10–20 mM) had a slight or no depressant effect. The concentration-response curve was very steep between 20 and 50 mM suggesting that even a small increase in the dose of urethane may profoundly affect reflex responses at spinal cord level. This assumption agrees well with our findings on the spinal somatovesical excitatory reflex in urethane anesthetized animals (see fig. 1).

Cross and Dyer[23] observed that the unit activity in rat diencephalon is unaffected by urethane anesthesia (1.3 g/kg i.p.) and concluded that urethane is suitable for neurophysiological investigation on the effect of substances on the hypothalamic activity. Similar conclusions were reached by Dyball and Mc Phail[30]. Cross and Silver[24] showed that in urethane-anesthetized rabbits (1 g/kg, 60% i.v., 40% i.p.) hypothalamic neurons respond to a variety of thermal, painful and auditory stimuli, as well as to hypoxia and hypercapnia. In urethane-anesthetized animals these stimuli increase blood pressure and produce a compensatory reflex bradycardia along with activation of the electrocorticogram[24]. These findings indicate that under urethane anesthesia peripheral stimuli are still able to activate structures at CNS level and produce reflex changes in autonomic functions.

Angel and coworkers studied the effects of urethane on the activation produced at various levels in the CNS by a peripheral sensory stimulation[1-7]. Angel and Unwin[7] described that urethane, even in sublethal doses, had no effect on the neuronal firing in the cuneate nucleus activated by the application of a somatic stimulus. Transmission to the ventrobasal thalamus and in a certain group of cortical cells was more sensitive to depression by urethane anesthesia. However, this effect occurred only at doses higher than those (1.25–1.5 g/kg i.p.) required for surgical procedures which allowed recording of neuronal activity.

Angel et al.[4] studied the effects of urethane on the cortical evoked response to stimulation of the forepaw. Progressive increase in anesthetic dose (starting from 1.25 g/kg i.p.) produced a dose-related increase in latency of cortical evoked response along with a depressed amplitude of the initial positive and negative waves. These results were interpreted as an indication that, with increasing depth of anesthesia, urethane: a) slows the transmission in the sensory volleys from the periphery to the cortex; b) decreases the size or dispersion of thalamocortical volley and c) decreases the effectiveness of this volley for triggering firing of the cortical cells[4]. All these effects of urethane were reverted by exposing the animals to high pressure, which suggests that they are related to induction of surgical anesthesia[2,4].

Administration of urethane to unanesthetized rats with implanted electrodes produced a dose-related change in the magnitude and latency of the cerebral evoked response to peripheral stimulation[5]. These observations led Angel to conclude that increased latency, and decreased amplitude, of cortical responses to peripheral stimulation represent the neurophysiological correlates of urethane-induced anesthesia[2,5].

Crawford and Curtis[22] found that in the 'cerveau isolé' preparation anesthetic doses of urethane depressed both spontaneous and stimulated firing of deep pyramidal neurons (including Betz cells) in the cat precruciate cortex.

Lincoln[44] described that urethane (1.2 g/kg i.p.) provides surgical anesthesia allowing constant EEG recordings for 8–12 h. The EEG pattern of urethane anesthetized animals closely resembles that of the normal sleeping cycle in the same species, indicating that this anesthetic does not produce marked depression of brain activity[44].

Very recently Pirch et al.[52,52a] showed that conditioned slow potential responses[52] and conditioning-related single unit activity[52a] recorded from the frontal cortex of urethane anesthetized rats (1.2–1.8 g/kg i.p.) do not differ from those recorded from unanesthetized animals. Accordingly urethane anesthesia appears to be suitable for neurophysiological experiments to investigate the mechanism(s) and pharmacology of learning[52,52a].

Taken as a whole these findings suggest that urethane anesthesia is characterized by a slight depressant effect on

neuronal function in various areas of the CNS. Depression of neurotransmission at the thalamic level[2,3,7] and certain cortical cells[22] is presumably relevant for induction of surgical anesthesia following urethane. On the other hand the relative lack (in anesthetic doses) of effects on transmission and spontaneous firing in some subcortical structures[7,23,24] are presumably relevant to account for the preservation of a number of autonomic reflex responses under urethane anesthesia.

Effect of urethane on neurotransmitter release

Only a limited amount of information is available about the effects of urethane on neurotransmitter(s) release. Larrabee et al.[42,43] showed that urethane has the unusual property (as compared to other anesthetics) of depressing, to a similar degree, both axonal conduction and synaptic transmission. These depressant effects were observed only at concentrations 5–15 times higher than those found in the plasma of urethane-anesthetized animals[34,42].

Halliday et al.[34] showed that urethane, in anesthetic concentrations, has no effect on acetylcholine release in the guinea pig myenteric plexus preparation. High concentrations of urethane (50–100 mM) have a depressant effect which, however, is not reverted by exposure to high pressure. Therefore it appears unlikely that depression of acetylcholine release is involved in producing urethane anesthesia. Urethane does not modify either uptake or release of GABA or aspartate from rat thalamic or cortical slices[39,49]. However, urethane anesthesia (0.89 g/kg i.p.) was found to produce a 30% decrease in spontaneous output of glutamate in the rat cerebral cortex[50].

Taken as a whole, these findings indicate that urethane anesthesia could be suitable for physiopharmacological studies concerning neurotransmitter release and its modulation by drugs. This assumption is easily verified when considering that urethane-anesthetized rats are commonly employed when studying acetylcholine release from the cerebral cortex[46,47].

In addition, urethane anesthesia appears to be suitable for studies involving neurotransmitter release in response to a physiological-like activation. For instance rabbits anesthetized with urethane i.p. (1.5 g/kg) have been used for studying the light-evoked release of tritiated acetylcholine (and its modulation by GABA) from the retina[25,48].

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