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Renin Release, an Artifact of Anesthesia and its Implications in Rats (38597)

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(Introduced by E. G. Erdos)

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High and markedly variable serum renin activity occurred in rats during previous investigations (1) while using pentobarbital or ether: These alterations of renin release were prevented by omitting the anesthesia. Moreover, these artifactual increases in renin activity previously have caused the rat to be considered as a unique species with regard to certain endocrine responses. Inconsistencies attributable to anesthetics have been found with regard to the sensitivity of the adrenal cortex to angiotensin II (2–4), deoxycorticosterone suppression of serum renin activity (1, 5) and apparent elevation of serum renin activity in the spontaneously hypertensive rat (6, 7).

Because of (a) the extensive biologic activities of angiotensin (see Discussion) and aldosterone, (b) the similarities of renin release in the rat to man (1, 8) and (c) the general investigative utility of the rat, a systematic attempt was made to find an anesthetic agent which would not release renin.

Materials and Methods. Male Wistar rats weighing 200–300 g were decapitated using a guillotine and the aortic blood was collected from the trunk for the first four seconds in iced plastic or siliconized glass tubes. Serum was removed following centrifugation at 4° and stored at −20° until radioimmunoassay for renin activity was performed. The method for measurement of serum renin activity (SRA) was previously described (1) with several modifications (9). In brief, serum was thawed on ice and treated with a 1 % solution of diisopropyl-fluorophosphate (DFP) in isopropyl alcohol followed by EDTA, and the pH was adjusted to 6.5–6.7 range with a citrate–HCl buffer solution. Serum samples (0.3–0.5 ml) were incubated at 37° for 2 or 3 hr and the samples cooled rapidly to 4°. Rate of production of angiotensin I (A_I), as an indicator of SRA, was quantified in the serum using a standard radioimmunoassay technique, the components of which were developed in our laboratories. Serum renin activity is expressed in the results as ng of A_I generated per 100 ml of serum per hour.

Radioimmunoassay of serum aldosterone level was performed by the method of Gomez–Sanchez *et al.* (10).

Parenteral anesthetic agents were administered intraperitoneally, and the volatile anesthetics were given through precalibrated specific vaporizers (E.M.O. Ether Inhalor for ether, Fluotec Mark-2 vaporizers for halothane, and Pentomatic vaporizer for methoxyflurane) into a glass chamber containing soda–lime, wherein rats were anesthetized in succession. Saline injections and air-insufflations into the same glass chamber were included for appropriate controls.

The depth of anesthesia was quantified for each anesthetic agent by testing loss of righting and pain reflex in experiments separate from the renin release studies. An example of these studies with ketamine is given in Fig. 1. The animals were considered "anesthetized" when both reflexes were lost. Doses of anesthetics and duration of anesthesia were chosen on this basis. Thus, in testing the qualitative capacity of anesthetic agents to induce renin release, the animals were decapitated when considered "anesthetized" by these criteria.

Systolic blood pressure was monitored in the rat tail using an automatic cyclic pneumatic pulse transducer (Narco Bio-Systems). The heart rate was calculated from the systolic pulse of the tail artery.

Rats were caged individually in an air-conditioned room with an automated lighting cycle coming on at 6:00 AM and off at 6:00 PM. They were given tap water *ad libitum* and Purina Rat Chow with sodium content of 152 mEq/kg.

Results. Intraperitoneal sham injection or saline injection did not induce significant renin release. That is, the control serum renin activity (SRA) was 90 ± 32 $ngA_I/100$ ml/hr; for sham injected animals it was 102 ± 36 and for saline injection 132 ± 61 $ngA_I/100$ ml/hr. Pentobarbital induced significant ($P < 0.01$) renin release (SRA = 554 ± 255 $ngA_I/100$ ml/hr). Each of the commonly used intravenous anesthetic agents induced highly significant ($P < 0.001$) renin release at 15–20 min after intraperitoneal administration (Fig. 2). Chloralose did not induce renin release at a high dose of 60 mg/kg nor did it induce anesthesia. Some ataxia was observed with chloralose but pain and righting reflexes were present. Morphine, the one narcotic agent studied caused a significant increase in renin release (Fig. 2).

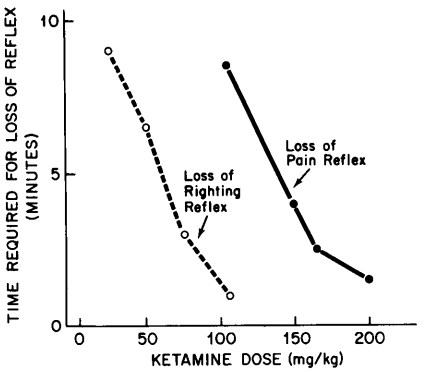

FIG. 1. Depth of anesthesia in the rat as determined by time for loss of reflex response for increasing doses of ketamine. Each point is the mean for six animals.

Commonly used inhalational anesthetics also induced significant ($P < 0.01$) renin release following 5 min of exposure to these agents (Fig. 3 and 4). Prolongation of the anesthetic time further increased the renin release as is shown with ether (Fig. 3). Halothane at a 1% concentration induced ataxia and loss of righting reflex but did not ablate the pain reflex. This 1% concentration did not induce significant elevation of SRA (Fig. 3). However, when used at 2%, the concentration of halothane required for reproducible anesthesia, significant ($P < 0.01$) renin release occurred. Renin release induced by diethyl ether was dose and time related (Fig. 3). Nitrous oxide, at a maximal concentration of 80%, did not induce significant renin release (Fig. 4), nor did it anesthetize the animals (righting reflex was partially impaired and still responded to pain). Methoxyflurane and cyclopropane also increased SRA (Fig. 4).

Following pentobarbital 30 mg/kg ip, SRA reached a peak at 20 min and gradually

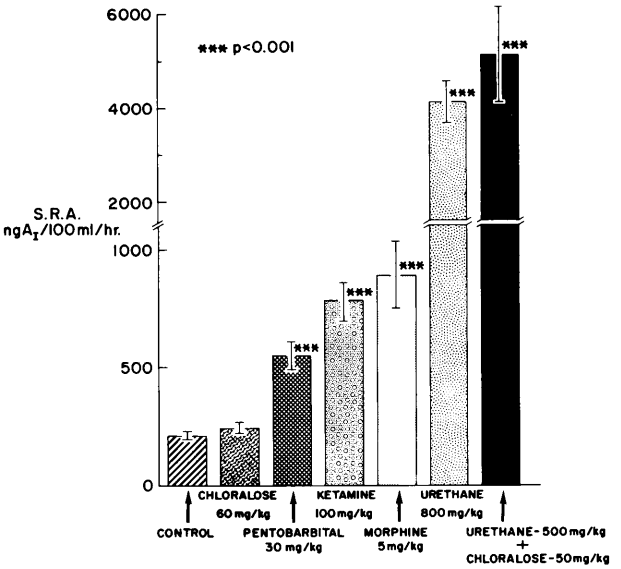

FIG. 2. Serum renin activity following various intravenous anesthetic agents in rats. Rats were sacrificed 15–20 min after intraperitoneal administration. Each bar represents mean ± SE of seven animals.

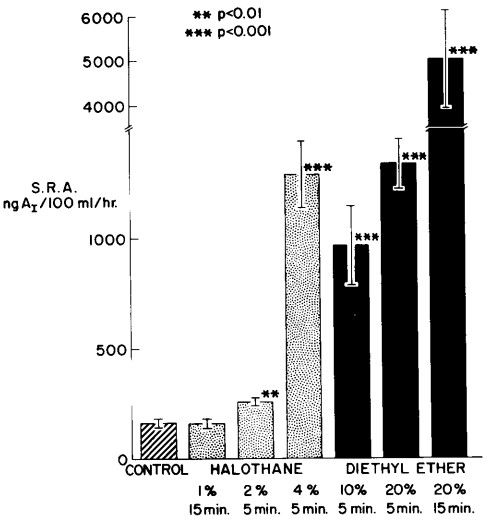

FIG. 3. Serum renin activity during inhalational anesthesia in rats. Rats were anesthetized in a soda-lime containing glass jar. Control rats received 5 min air insufflation in the same jar. Each bar represents mean ± SE of seven animals.

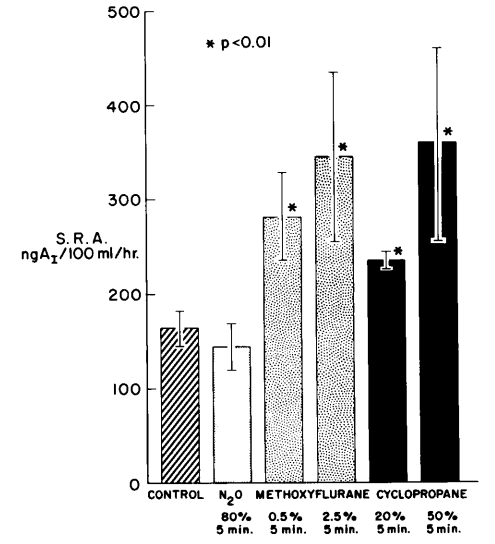

FIG. 4. Serum renin activity during inhalational anesthesia in rats as in Fig. 3. Each bar represents mean ± SE of seven animals.

declined to below the control level at 100 min (Fig. 5). The onset and loss of anesthesia was parallel to this elevated SRA. The well-known acute reduction in blood pressure was noted in addition to an increased heart rate.

Urethane 800 mg/kg induced highly significant ($P < 0.001$) renin release which was sustained at remarkable levels as long as 100 min (Fig. 6). Blood pressure and heart rate tended to rise but these changes were not statistically significant.

Highly significant renin release ($P < 0.001$) occurred with urethane, 800 mg/kg ip and 400 mg/kg ip, which were significantly ($P < 0.001$ and $P < 0.05$) impaired by pretreatment with propranolol 1.5 mg/kg s.c. (Fig. 7), and were accompanied by significant ($P < 0.01$) rise in serum aldosterone level (Fig. 8). Propranolol pretreatment gave equivocal results as to blockade of renin release with pentobarbital and morphine (24% and 19% inhibition, respectively). This increase in serum aldosterone level induced by urethane was also significantly ($P < 0.01$) impaired by prior treatment with propranolol 1.5 mg/kg (Fig. 8).

Discussion. The induction of a surgical plane of anesthesia and induction of renin release in the rat are parallel or simultaneous events. Angiotensin has a broad spectrum of pharmacologic activities, particularly in the central and peripheral autonomic nervous system (11–16) along with a hormonal role in aldosterone release. Thus, the increment in angiotensin formed from anesthesia-induced renin release in rats would be expected to cause artifacts in cardiovascular and endocrine investigations.

Pentobarbital anesthesia induces renin release in dogs (17) suggesting that the rat species is not unique. This cause and effect relationship is less clear in humans (18) in that significant renin release during anesthesia occurred primarily in patients with a predisposing factor such as volume depletion, blood loss etc.

Anesthesia-induced renin release can mediate cardiovascular effects. For example, a unique interrelationship between ketamine-induced renin release and its pressor role was previously delineated (19). However, a pressor role for anesthesia-induced renin release is not clear with the two anesthetic agents studied here. For example, pentobarbital (Fig. 5) initially induced a decline in blood pressure and a 20% increase in heart rate. Urethane anesthesia (Fig. 6) induced extremely high renin activity but only a mild blood pressure elevation. Volicer and Loew

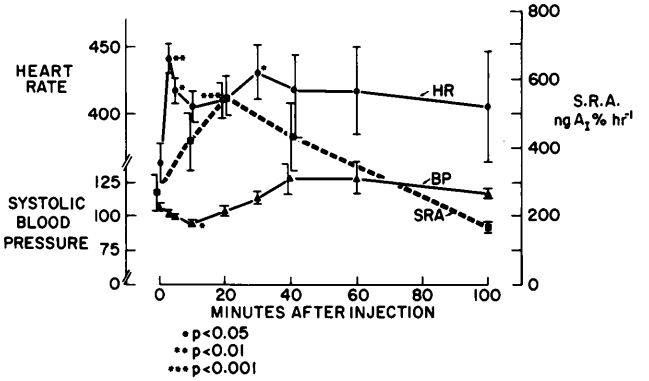

FIG. 5. Blood pressure, heart rate, and serum renin activity with pentobarbital 30 mg/kg ip. Each point represents mean ± SE of six animals.

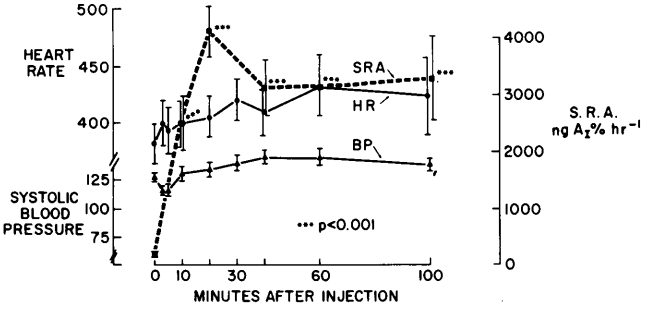

FIG. 6. Blood pressure, heart rate, and serum renin activity with urethane 800 mg/kg ip. Each point represents mean ± SE of six animals.

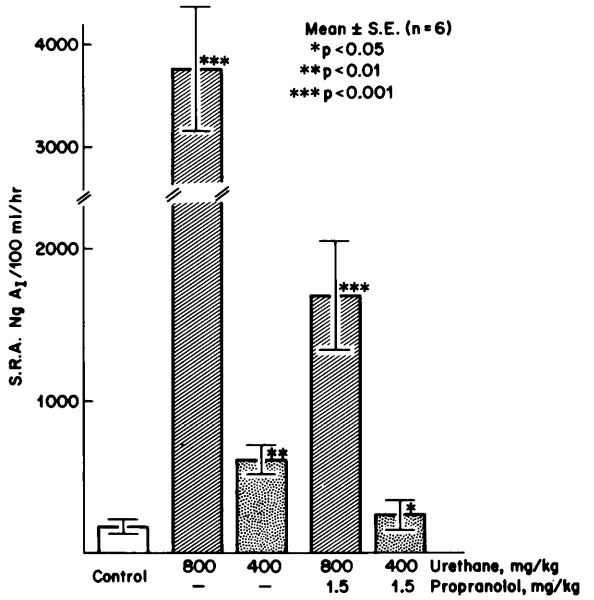

FIG. 7. Effects of propranolol on urethane-induced renin release. Rats were pretreated with subcutaneous propranolol 15 min prior to urethane administration. Rats were sacrificed 20 min after intraperitoneal saline or urethane injection. Each bar represents mean ± SE of six rats.

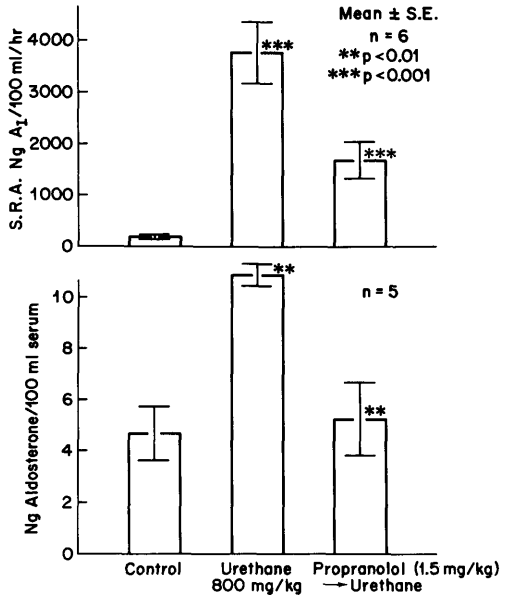

FIG. 8. Effects of propranolol on urethane-induced renin and aldosterone release. Propranolol was given 15 min prior to urethane. Rats were sacrificed 20 min after saline or urethane. Each bar in S.R.A. represents mean ± SE of six rats and in aldosterone level of five rats.

(20) noted a decreased response to exogenously administered angiotensin in urethane anesthetized rats. However, since endogenous angiotensin was probably at high levels, small exogenous angiotensin doses could be relatively inactive.

Studies utilizing "light-ether" anesthesia have attributed a unique quality to renin release in the rat (Goodwin *et al.* 5, Cade and Perenich, 2) as well as implying high renin as a possible cause of hypertension in young SH rats (Sen *et al.*, 7). Goodwin *et al.* (5) reported that deoxycorticosterone acetate and sodium chloride (DOC-Na) did not suppress renin release in the "light-ether" anesthetized rat. However, anesthesia overrides the renin suppression effect of DOC-Na (1) and thus their result was clearly an artifact of the anesthesia *per se*. Cade and Perenich (2) found a reduced sensitivity of the adrenal cortex to infused angiotensin in the ether anesthetized rat. When anesthesia was omitted, a 1000-fold increase in senitivity could be demonstrated, indicating that anesthesia obscured the angiotensin-induced increment in aldosterone release (3, 4). Sen *et al.* (7) have found higher SRA in SH rats than in normotensive rats when both groups were subjected to "light-ether" anesthesia. However, the SRA in control rats was already 10–15 times higher than SRA when the values are compared to those from unanesthetized rats (1). Other investigators (6) have not found elevated SRA in SH rats at various ages when unanesthetized rats were used. Consequently, the higher SRA in SH rats reported by Sen *et al.* (7) may be a poorly understood effect of this anesthetic *per se*.

Diethyl ether, one of the most commonly used anesthetic agents in laboratory animals, released large quantities of renin even when relatively low concentrations of 10% and 20% (controlled saturations) were used for only 5–10 min. Since the vapor pressure of ether at 20° is 450 mm Hg, the concentration of ether-saturated air is well above 50%. With these high ether concentrations anticipated from uncontrolled administration, very high SRA would be expected. Hence, the concept of "light-ether" anesthesia as a method for minimizing anesthetic artifacts is fallacious.

There is considerable evidence showing that nerve-stimulated (21), stress-induced (8), catecholamine-induced (22, 8) and vasodilatory drug-induced (9) renin release are significantly impaired by propranolol pretreatment. In two instances, ketamine (19) and urethane, anesthesia-induced renin release is impaired by pretreatment with propranolol and this would appear to be partially mediated by beta-adrenergic receptors.

Urethane-induced (and possibly other anesthesia-induced) renin release was accompanied by elevated serum aldosterone levels, and this aldosterone release was also impaired by prior treatment with propranolol. These data suggest that a high endogenous angiotensin level does initiate aldosterone release in the rat and reinforce the importance of considering endocrine artifacts due to anesthesia. Anesthetic agents are also known to cause significant ACTH release (23), and ACTH can acutely stimulate aldosterone release.

In conclusion, each of the commonly used anesthetic agents induced renin release in

the rat. The angiotensin formed in serum from the elevated SRA has the potential for extensive pharmacologic effects (11–16, 24, 25). It is possible that control renin samples drawn soon after administration of an anesthetic (during ataxic phase) and/or with lower doses (e.g., 20 mg/kg in the case of pentobarbital) may not exhibit grossly elevated values as compared to unanesthetized controls. However, artifactual changes due to anesthesia *per se* should be thoroughly investigated if the technique of quick sampling or lower doses is to be used. The effect of anesthetic agents in other species has not yet been systematically evaluated except for a limited study reported in man (18) and in the dog (17).

Summary. In our attempt to find an anesthetic agent which did not influence the renin–angiotensin system in the rat, the effect of widely used injectable and gaseous anesthetics and narcotic agents on renin release was characterized. All of the agents studied induced dose- and time-related increases in serum renin activity when administered in anesthetic doses. Preliminary experiments indicated that cardiovascular effects were highly variable, giving little insight into the relationship between renin release and cardiovascular changes. Propranolol impaired most of the anesthesia-induced renin release and impaired aldosterone release with the one agent (urethane) studied.

Renin release by two anesthetic agents (ketamine and urethane) appeared to be mediated primarily through the beta-adrenergic receptor mechanism, but equivocal results were obtained with other agents (pentobarbital and morphine). It is possible that other anesthetics, as with urethane, may induce aldosterone release by way of renin release. This anesthesia-induced renin release and the extensive biologic activities of angiotensin and aldosterone suggest a potential for influencing many investigations, particularly those involving cardiovascular and endocrine systems.

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