Long-Term Effects of Pentobarbital Anesthesia on the Atrial Natriuretic Peptide System in Rats (43678)

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Abstract. **A long-term effect of pentobarbital treatment on the storage and release of atrial natriuretic peptide (ANP) was investigated in rats. The experimental group was treated with pentobarbital (50 mg/kg, ip), and the control group was injected with the vehicle only. They were used one week after the treatment. Male and female rats were used separately to see if there exists a sex difference in the response to pentobarbital. In male rats, the plasma ANP measured in a conscious state was significantly higher in the experimental group than in the control. The ANP content as well as the number of specific granules in the atrial tissue was significantly lower in the experimental group. In response to extracellular volume expansion (VE), amounting up to 5% of the body weight over 30 min, while the plasma ANP increased in the control, it did not significantly change in the experimental group. Urinary responses to the exogenous infusion of ANP did not differ in magnitude between the two groups. In female rats, neither the plasma level nor the atrial content of ANP (or the atrial granularity) was different between the experimental and control groups. Nor did the increase of plasma ANP in response to VE differ in magnitude between the two groups in female rats. These results indicate that pentobarbital may have a long-term effect on the storage and release of ANP.** [P.S.E.B.M. 1994, Vol 205]

General anesthesia affects bodily responses to various physiological and pharmacological stimuli (1). Anesthetics may influence the release of certain hormones (among others) affecting cardiovascular functions. The stimulatory effect of some anesthetics, such as ether, pentobarbital, ketamine, urethane, and chloralose, on renin secretion is well known (2–5). Some may inhibit the release of catecholamines (6). As with other hormones, it may be plausible to hypothesize that anesthetics may influence the secretion of atrial natriuretic peptide (ANP). Horky *et al.* (7) and Gutkowska *et al.* (8) have indeed found that anesthesia changes the plasma level of ANP.

It has been found that the metabolites of pentobarbital are slowly eliminated in the urine over several days following its administration in humans (9). It may then be speculated that pentobarbital remains in the heart over a prolonged period of time and affects the storage and release of ANP. Whether pentobarbital chronically affects the ANP system has not, however, been documented.

The present study was aimed at investigating a long-term effect of pentobarbital treatment on the storage and release of ANP. Atrial morphology was simultaneously determined to see the degree of atrial granularity. Whether there exists a sex difference in the response to pentobarbital was also explored.

Methods

Animals and Groups. Sprague-Dawley rats weighing 200–240 g were used. They were maintained in accordance with standards of care and use recommended by the American Physiological Society. They were housed in a temperature- and light-controlled en-

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vironment and had free access to food and water until used.

The experimental group was treated with pentobarbital (*Nembutal,* Abbott Laboratories; 50 mg/kg, ip), and the control injected with the vehicle only (40% propylene glycol and 10% ethanol in water). They were used one week after the treatment. Male and female rats were used separately, so that overall four groups were prepared.

Experimental Protocols. In the first series of experiments, plasma concentrations of ANP and renin were determined in a conscious state. The animals were killed by decapitation and trunk blood was collected in ice-cold tubes containing 200 μl of a mixture of ethylenediaminetetraacetic acid (EDTA, 1 mg/ml of blood), phenylmethylsulfonyl fluoride (50 m*M*), aprotinin (1,000 KIU/ml), and soybean trypsin inhibitor (SBTI, 50 BAEE/ml). The right and left atria were removed separately.

In the second series of experiments, the release-responsiveness of ANP was examined. One week following the pentobarbital (or vehicle) treatment, on the experimental day rats were anesthetized with thiopental (50 mg/kg, ip) and were subjected to extracellular volume expansion (VE). The right femoral artery was cannulated to measure the arterial blood pressure. The vein was catheterized, through which VE was induced by infusion of iso-oncotic saline (7 g albumin/100 ml 0.9% NaCl) over 30 min, total volume infused amounting up to 5% of the body weight. Blood samples were taken from the artery before and after VE.

In the third series, urinary responses to exogenous infusion of ANP were examined under thiopental (50 mg/kg, ip) anesthesia in the experimental and control groups of male rats. Urine samples were collected through a bladder catheter before and during the infusion of ANP (atriopeptin III; Sigma Chemical Co., St. Louis, MO; 300 ng/15 μl/min). The infusion was continued for 30 min. Upon termination of the infusion, blood samples were taken from the artery. Urinary sodium concentration was measured by flame photometry.

Radioimmunoassay of ANP and Renin. The blood sample was centrifuged at 4°C, and the plasma kept at −70°C until analyzed. The plasma was thawed, extracted with Sep-Pak C18 cartridges (Waters Associates, Milford, MA) and lyophilized. It was then reconstituted with Tris-acetate buffer (0.1 *M,* pH 7.4, containing 0.2% neomycin, 10 m*M* EDTA, 50 BAEE/ml SBTI, 0.02% sodium azide, 200 KIU/ml aprotinin, and 1% bovine serum albumin). Atrial tissue was boiled in 1.0 *M* acetic acid for 10 min, homogenized, and centrifuged. The supernatant was diluted with Tris-acetate buffer.

Concentrations of ANP in the aliquots were determined using radioimmunoassay kit (Research & Diagnostic Antibodies, Berkeley, CA). The determined values were corrected with the extraction ratio (68.5 ± 1.4%). Plasma renin concentration (PRC) was also measured by radioimmunoassay using unextracted plasma as described previously (10).

Atrial Morphology. The right atria were removed from the decapitated animals. The tissue specimens were obtained at the same atrial region from each animal. They were fixed for 2 hr in glutaraldehyde (6.7%) in cacodylate buffer (0.1 *M*) containing paraformaldehyde (1.6%). They were then post-fixed for 2 hr in osmium tetroxide (1%), dehydrated in a series of alcohols and embedded in Epon mixture (Polysciences, Warrington, PA). Thin sections stained with uranyl acetate and lead citrate were examined electron microscopically (JEOL JEM 1200EX).

A quantitative method was used to determine atrial granularity. The longitudinally sectioned cardiocytes containing nucleus were photographed at an original magnification of ×3,000, and analyzed with a final magnification of ×5,000. In each cardiocyte, perinuclear granules within the grid (area of 20 × 10 μm^2) were counted. To match the sectioned level of the cardiocytes between the groups and to justify that the counted sections represent comparable sites, the nuclear size was also measured.

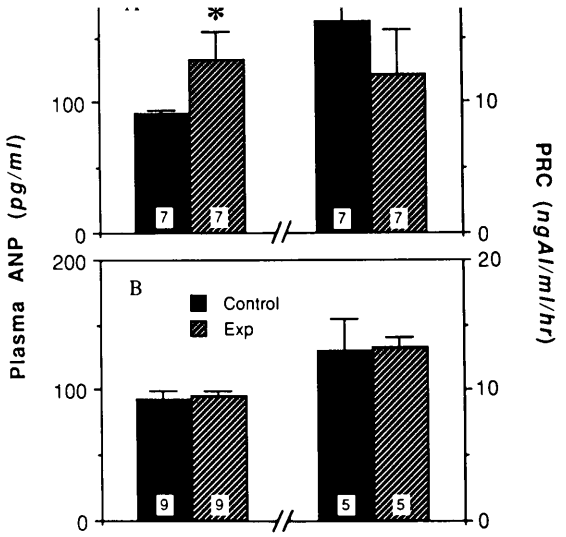
Statistics. Data are expressed as mean ± SEM. The statistical significance was determined using BMDP 2V software (11). ANOVA with two groupings and one within factor was applied for the VE study. Two-way ANOVA or t-test was used for the others.

Results

Plasma Concentrations and Atrial Contents of ANP. Figure 1 shows plasma concentrations of ANP and renin in the conscious state, and Figure 2 the right and left atrial tissue contents of ANP. In male rats, the plasma ANP was significantly higher and the tissue content lower in the experimental group than in the control. PRC did not significantly differ between the two groups.

In female rats, neither the plasma ANP nor PRC was significantly different between the experimental and control groups (Fig. 1). Nor did the ANP content differ in the right and left atria (Fig. 2).

Atrial Morphology. Atrial granules appeared in either direction from the poles of the nucleus, in association with the Golgi complex. In male rats, they numbered significantly less in the experimental group than in the control (Figs. 3 and 4). Apart from the difference in granularity within the cardiocyte, no obvious qualitative difference was noted in overall atrial morphology, i.e., there was no evidence of cardiomyopathy due to pentobarbital (Fig. 3). In female rats, the number of atrial granules did not differ between the experimental and control groups (Fig. 4).



ıre 1. Plasma atrial natriuretic peptide and renin concentra- s in male (A) and female (B) rats. [Exp] denotes the group ch had been treated with pentobarbital one week before)d sampling. ANP, atrial natriuretic peptide; PRC, plasma n concentration. Numerals indicate numbers of rats in each ıp. *$P < 0.01$, compared with the control (non-paired t-test).

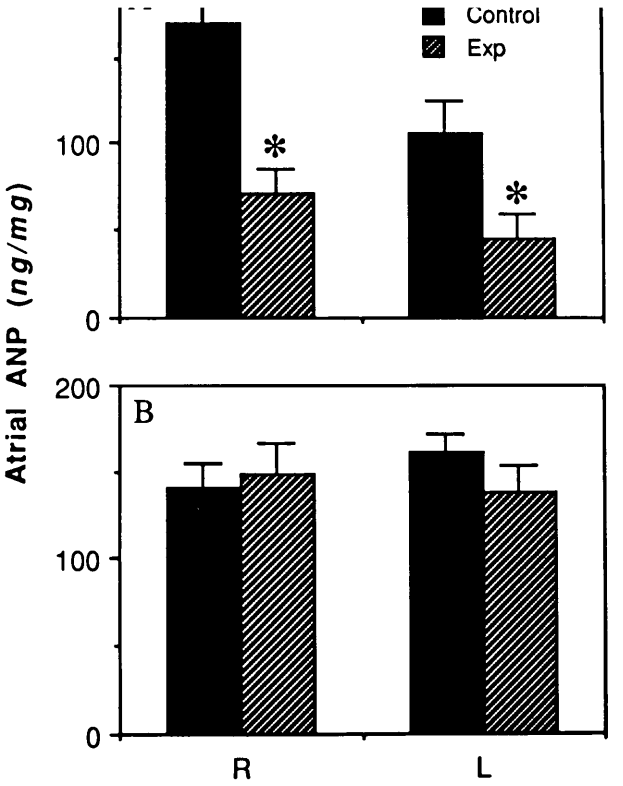


Figure 2. Right (R) and left (L) atrial tissue contents of ANP in male (A) and female (B) rats. Legends as in Fig. 1.*$P < 0.01$, compared with the control (non-paired t-test). Interaction between groups and sexes was statistically significant (right atria, $P < 0.01$; left atria, $P < 0.05$; two-way ANOVA).

The nuclear area in the counted field ($20 \times 10\ \mu m^2$) s comparable between the groups in both sexes g. 4).

ANP Responses to Volume Expansion. Figs. 5 l 6 depict the plasma ANP values before and after in the male and female, respectively. In the male, ile the plasma ANP increased in the control (from 4 ± 4.6 to 78.7 ± 4.3 pg/ml), no significant change s noted in the experimental group (from 42.1 ± 5.0 37.0 ± 2.4 pg/ml). In the female, VE increased the sma ANP in the experimental group (from 53.1 ± to 77.9 ± 8.9 pg/ml) as much as in the control (from 6 ± 1.6 to 69.4 ± 3.5 pg/ml). Blood pressure was ferent during VE between the two groups neither in male nor in the female.

Urinary Responses to ANP Infusion. Urinary ume and sodium excretion were increased in re-)nse to the exogenous infusion of ANP in both ex- imental and control groups of male rats. The degree the increases did not differ between the two groups: ume increased from 8.2 ± 0.5 (basal) to 38.3 ± 10.9 min (during the second 15-min period of the infu- n) in the experimental group ($n = 6$) and from 7.5 ± to 28.7 ± 7.2 in the control ($n = 6$); sodium excre- n from 0.18 ± 0.03 to 3.04 ± 0.96 μEq/min in the perimental group and from 0.25 ± 0.03 to 3.73 ± 3 in the control.

Plasma ANP concentrations measured upon ter- nation of the infusion were comparable between the perimental and control groups (194.5 ± 9.1 vs 174.8 17.6 pg/ml).

Discussion

The present study indicates that pentobarbital may chronically affect the ANP system. Male rats treated with pentobarbital one week previously showed a higher basal plasma ANP compared with the control rats treated with the vehicle only, in association with a diminished tissue content of ANP in both atria and a reduced atrial granularity.

The decreased tissue storage of the hormone may reflect either a reduction in synthesis or an increase in basal release from the storage site into the circulation. The elevated plasma ANP coinciding with the depleted storage (the diminished atrial tissue contents and granularity) would suggest that the latter is more likely, as has been suggested by previous authors (12).

Alternatively, the elevated plasma level may have resulted from a decreased removal from the circulation. Urinary responses to the exogenous ANP, however, were not affected by the previous pentobarbital treatment, and the plasma ANP measured upon discontinuation of the infusion was comparable in the two groups. These findings suggest that pentobarbital does not affect metabolism or clearance of ANP from the circulation.

In response to extracellular VE, while the plasma

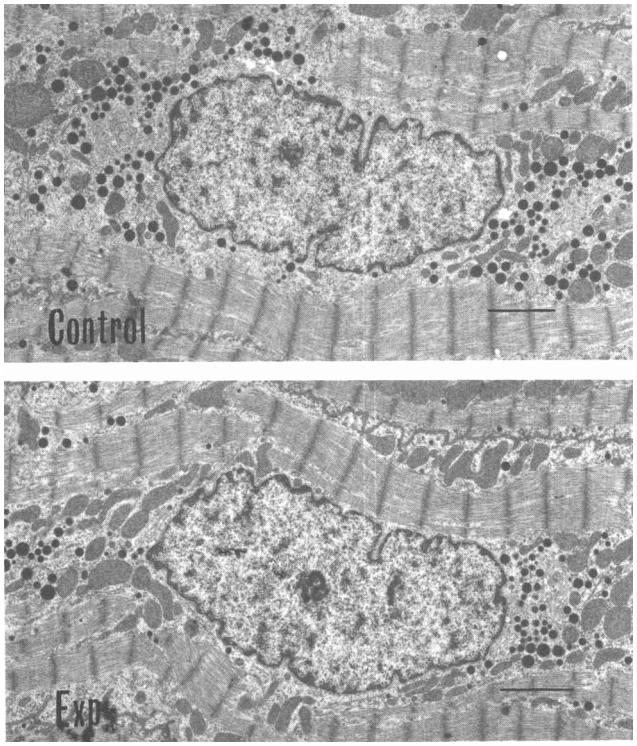


Figure 3. A cardiocyte of the right atrium from control and experimental groups of male rats. Magnification is ×2,800; bar 2 μm.

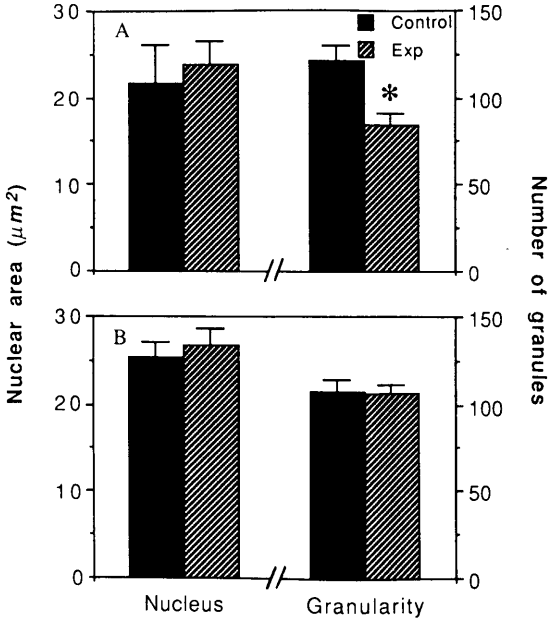


Figure 4. Comparison of nuclear area and number of granules in the right atria from control and experimental groups of male (A) and female (B) rats. Each column represents the mean of 18–23 counts. *$P < 0.01$, compared with the control (non-paired t-test).

ANP increased in the control, it did not significantly change in the experimental group. The attenuated responsiveness to a given stimulus in the experimental group may be attributed to the diminished tissue reserve.

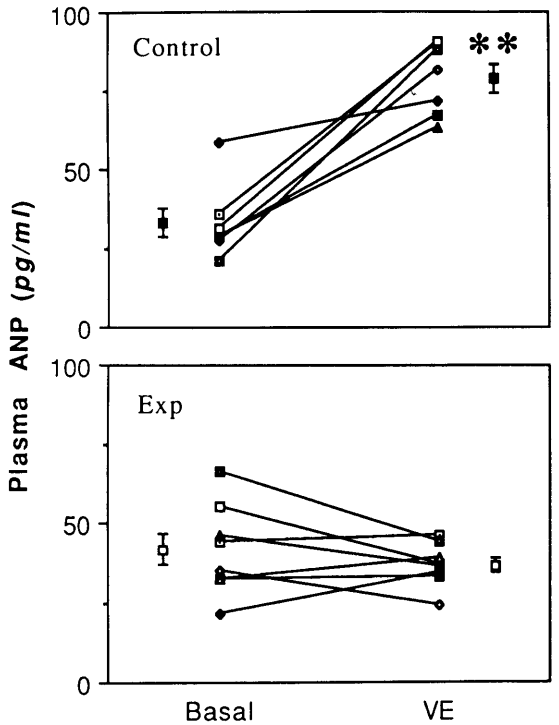


Figure 5. Plasma ANP concentrations before (basal) and after volume expansion (VE) in male rats. Individual and mean ± SEM values are shown. Legends as in Fig. 1. **$P < 0.01$; compared with the basal (paired t-test).

The mechanism underlying the long-term effect of pentobarbital on the ANP system is not clear. The increased basal plasma level may indicate an expansion of the circulating volume. Effects of pentobarbital on the plasma volume is not well determined (13, 14), however. While pentobarbital anesthesia is commonly associated with an antidiuresis caused by enhanced release of vasopressin (15), this does not completely explain the sustained fluid retention. It is also unlikely that a hemodynamic change is responsible for the altered ANP response, since blood pressure was different between the experimental and control groups neither before nor during VE.

Yet one possibility that could account for the increased basal level is the stress, since there is evidence that ANP is released under stress (8, 16). If the pentobarbital-treatment renders more stress than the vehicle-injection, then a higher plasma ANP may be expected.

Another possibility still remaining to be verified is that pentobarbital itself or its metabolites may directly affect the ANP system. In humans, the metabolites of pentobarbital are slowly eliminated in the urine over five days following the administration (9). Pentobarbital (or its metabolites) may remain in the heart over a prolonged period of time, affecting the storage and release of ANP. Further studies will be needed to clarify the point.

Interestingly, a sex difference was noted in the

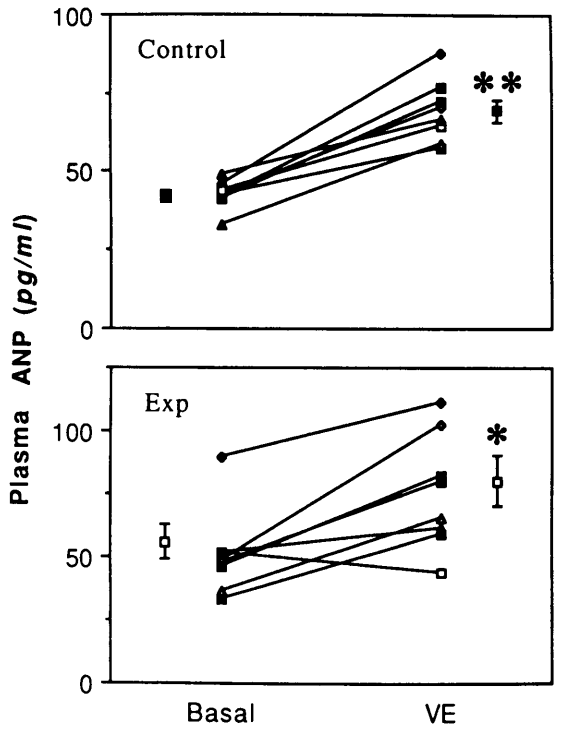


Figure 6. Plasma ANP concentrations before and after VE in female rats. Legends as in Fig. 5. $^{*}P < 0.05$, $^{**}P < 0.01$; compared with the basal (paired t-test). When Figs. 5 and 6 were taken together, a significant interaction of the group difference with sexes was noted ($P < 0.001$, ANOVA with two groupings and one within factor).

ANP response to pentobarbital. Neither the plasma level nor the tissue content of ANP (or granularity) was affected by the pentobarbital treatment in the female. Nor did the magnitude of the increase of plasma ANP in response to VE differ in the two groups of female rats. The mechanism for the sex difference has to be further evaluated.

Finally, the ANP response to pentobarbital cannot be attributed to a nonspecific effect on the hormonal system, since PRC was not affected even in the male.

In summary, we have found that the basal plasma ANP was elevated in the male rats treated with pentobarbital one week previously, in association with diminished atrial granularity and tissue contents of ANP. These rats also demonstrated an impaired release of ANP in response to an acute extracellular VE, which may be attributed to the diminished tissue reserve. In female rats, no such changes were noted, revealing a sex difference. These results suggest that pentobarbital has a long-term effect on the ANP system.

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