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The Role of Endorphins and Vasopressin in Canine Endotoxin Shock[1]

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Chemical antagonists were used to assess the role of β-endorphin and arginine–vasopressin (AVP) in canine endotoxin shock. Fifteen awake dogs were given *Escherichia coli* endotoxin IV. Within 5 min, CO decreased to 28%, LV *dP/dt* to 46%, and MAP to 52% of baseline. Fifteen minutes after endotoxin, five dogs each received naloxone, AVP antagonist, or no treatment. Control (untreated) animals exhibited persistent cardiovascular depression, with CO 49%, LV *dP/dt* 69%, and MAP 91% of baseline after 45 min. Naloxone improved CO to 69%, LV *dP/dt* to 94%, and MAP to 91% by 30 min after treatment. AVP blockade improved CO to 105%, LV *dP/dt* to 107%, and MAP to 95% of baseline by 30 min after treatment, and caused significant tachycardia. Plasma cortisol and AVP increased markedly in all groups after endotoxin administration. AVP antagonist treatment increased mean survival from 1.4 to 4 days. These data suggest that abnormally elevated AVP contributes to cardiovascular depression in canine endotoxin shock and that AVP blockade is therapeutic in the animal model studied.

INTRODUCTION

Endotoxin administration in dogs causes profound cardiovascular collapse, characterized by systemic hypotension and low cardiac output [17]. Although the mechanism of this response is not completely understood, a variety of factors including hepatic venoconstriction, reduced systemic venous return, circulating myocardial depressant factor, and direct myocardial effects, have been implicated [16, 18, 19, 25, 34]. Opiate antagonism with naloxone has been found to increase survival and improve cardiac performance in rats and dogs during endotoxin shock [7, 35, 36]. Since opiates exert a centrally mediated myocardial depressant effect [24], these studies suggested that endogenous opiates (endorphins), known to be elevated in septic shock [5], might contribute to hemodynamic deterioration.

Plasma levels of arginine–vasopressin (AVP) also increase markedly in dogs during endotoxin or hemorrhagic shock, much more than occurs during water deprivation [28, 44]. Furthermore, AVP has been shown to cause myocardial depression in dogs, both *in vivo* and in an isolated left ventricle preparation [29, 43]. These observations suggested the possibility that excessively high AVP activity in endotoxin shock might contribute substantially to cardiac depression. Our study was designed to test this hypothesis by treating endotoxin shocked dogs with a specific AVP antagonist and comparing the observed hemodynamic changes and survival with untreated control animals. Because of potential interactions between AVP and β-endorphin, a third group of naloxone-treated dogs was studied to allow a comparison with AVP antagonist effects.

METHODS

Fifteen mongrel dogs of both sexes (19–33 kg), free of dirofilaria and endoparasites, were briefly anesthetized (thiamylal sodium) for catheter placement 1 day prior to the actual experiment. Using sterile surgical technique, a cervical incision was used to insert a trans-

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jugular Swan–Ganz thermodilution catheter in the pulmonary artery. A carotid artery catheter and a transcarotid left ventricular pigtail catheter were inserted through the same incision. After the catheters were secured in place, the animals were allowed to recover from anesthesia and given free access to food and water. The animal research protocol followed in this study was approved by the Subcommittee for Animal Research, Ann Arbor Veterans Administration Medical Center.

One day after catheter placement, fully awake and normal-appearing animals were placed in a modified Pavlov stand. Mean systemic arterial pressure (MAP) and left ventricular (LV) pressure were continuously recorded. Heart rate was measured with an amplifier triggered by the LV pressure pulse. The maximum value during the cardiac cycle of the first derivative of LV pressure (dP/dt max, hereafter, dP/dt) was electronically derived from the LV pressure signal. Cardiac output (CO) and temperature were intermittently recorded using the thermodilution Swan–Ganz catheter. Hemodynamic variables were analyzed and reported at 15-min intervals during the 3-hr observation period, in addition to measurements made 5 min after endotoxin administration and 5 min after drug treatment. Stroke volume (SV) was calculated as SV = CO/HR (ml/beat). Total peripheral resistance (TPR) was estimated as TPR = MAP/CO [(mm Hg/liter/min) $\times$ 80 = dyne $\cdot$ sec $\cdot$ cm^{-5}], under the assumption that venous pressure contributed negligibly to the result. Blood samples obtained through the carotid artery catheter were replaced with an equal volume of normal saline.

After the awake dogs had stabilized for 30 min, baseline hemodynamic measurements and blood samples were obtained. Animals were then given *Escherichia coli* endotoxin intravenously (0.75 mg/kg IV, Sigma No. L-3124, serotype 0127:BB, phenol extracted), an LD_{100} dose at 48 hr in pilot studies. Fifteen minutes after endotoxin administration, treatment was initiated: five *control* dogs received only normal saline, 2 ml/kg, iv; five animals received *naloxone*, 2 mg/kg iv + 2 mg/kg/hr iv for 3 hr; five additional dogs received the *AVP antagonist* 1-(β-(mercapto-β, β-cyclopentamethylenepropionic acid), 2-(0-methyl) tyrosine arginine-vasopressin (Bachem, Inc.), 10 μg/kg iv [22]. These doses were selected because of demonstrated efficacy in previous studies [36, 37]. Both naloxone and AVP antagonist were dissolved and administered in saline, 2 ml/kg. All animals were monitored for 180 min after endotoxin administration and received normal saline, 1 ml/kg/hr, during this period. After this observation period, the dogs were returned to their cages and allowed free access to food and water, but were not treated with antibiotics or iv fluids. Catheters were removed under brief thiamylal sodium anesthesia on the day following the experiment. Survival was recorded for 7 days, at which time any surviving animal was sacrificed.

Blood samples at baseline, 30 and 90 min after endotoxin administration were obtained. White blood cell (WBC) count with differential, platelet count, and hematocrit were determined. Ionized calcium was measured from heparinized blood immediately after sampling (Orion SS-20 Ionized Calcium Analyzer). Total hemolytic complement was assessed by the percentage lysis of sensitized sheep red blood cells (RBC) by dog serum. Lysis of 60×10^6 RBC by a 1:40 dilution of dog serum after 1 hr incubation at 37°C was compared to standard (100%) lysis by distilled water using optical density at 540 nm. Plasma epinephrine, norepinephrine and cortisol were measured by radioimmunoassay [23, 32]. Plasma AVP was measured by radioimmunoassay from samples extracted using Sep pak C_{18} cartridges (Waters Associates). Separation of free from bound AVP was achieved by adding rabbit γ-globulin and polyethylene glycol [33]. Vasopressin antagonist was tested for cross reactivity in the AVP radioimmunoassay system. The antagonist showed a 0.46% cross reactivity with AVP standard.

Hematologic variables were compared within treated groups at baseline, 30 and 90 min by paired Student's t test, and between treatment groups by non-paired Student's t

TABLE 1

HEMODYNAMIC PARAMETERS AT BASELINE

	Treatment				
	Control	Naloxone	Vasopressin antagonist	*F* value	*P* value
Cardiac output (liter/min)	4.2 ± 0.4	3.8 ± 0.8	2.7 ± 0.3	2.5	0.13
MAP (mm Hg)	112 ± 3	117 ± 7	113 ± 6	0.2	0.80
Heart rate (beats/min)	110 ± 11	90 ± 9	111 ± 14	1.0	0.41
LV *dP/dt* (mm Hg × 10^3/sec)	3.1 ± 0.3	2.8 ± 0.3	3.1 ± 0.3	0.2	0.79
Stroke volume (ml/beat)	40 ± 4	44 ± 9	26 ± 4	2.1	0.16
TPR (dyne · sec · cm^{-5})	2240 ± 240	2800 ± 480	3520 ± 400	2.6	0.11
Temperature (°C)	39.4 ± 0.1	40.0 ± 0.2	39.4 ± 0.2	2.9	0.10

Note. $\bar{x} \pm SEM$ *F*, *P* values between groups by analysis of variance. Abbreviations: MAP, mean arterial pressure; LV *dp/dt*, left ventricular pressure-time derivative; TPR, total peripheral resistance.

test. Hemodynamic parameters were compared between treatment groups and across time by multiple analysis of variance. These data were then expressed as the ratio to initial baseline measurement, to eliminate large interanimal variations.

RESULTS

Baseline hemodynamic parameters showed no statistically significant variance between treatment groups prior to endotoxin administration (Table 1). Apparent differences were due to large baseline variation among all dogs rather than systematic differences between drug treatment groups that were randomly assigned. Immediately after endotoxin administration at *T* (time) = 0, CO fell to 28% of its initial level (Fig. 1). After this initial decrease, there was a gradual improvement in control animals, so that CO achieved 60% of its baseline level by 90 min and remained at this level during the subsequent 90 min observation period. Naloxone-treated animals demonstrated a more rapid improvement in CO than control animals, with a return of CO to 80% of baseline within 5 min of treatment ($T = 20$ min). The difference between naloxone-treated and control animals ($P = 0.08$) gradually diminished, so that CO in both groups was 65–70% of baseline by $T = 180$ min. Animals treated with AVP antagonist showed a rapid normalization of CO, within 10 min of treatment. CO re-

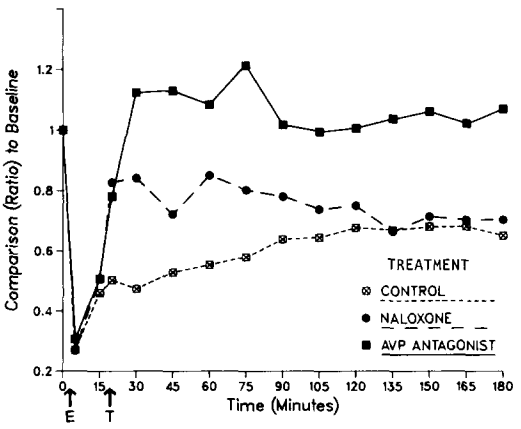

FIG. 1. CO fell markedly after endotoxin (E), but returned to normal after drug treatment (T) with AVP antagonist, significantly better than control ($P < 0.01$) or naloxone ($P < 0.03$) groups.

mained greater than baseline throughout the experiment, being significantly greater than control ($P < 0.01$) or naloxone-treated ($P < 0.03$) dogs.

LV *dP/dt* decreased to 46% of baseline immediately after endotoxin administration (Fig. 2). After treatment with naloxone and AVP antagonist, LV *dP/dt* returned to baseline level within 5–10 min. These drug treatment groups were statistically comparable, but were both different from control dogs, where LV *dP/dt* returned gradually toward normal only after 120 min (naloxone > control, $P < 0.01$; AVP antagonist > control, $P < 0.05$).

All dogs experienced tachycardia, beginning approximately 20 min after endotoxin administration (Fig. 3). AVP antagonist treatment was associated with the most rapid and profound tachycardia, beginning immediately after treatment and reaching a level 1.8 times baseline after 60 min. Tachycardia in AVP antagonist treated dogs was significantly greater than control or naloxone-treated groups ($P < 0.05$). Although naloxone-treated dogs appeared to develop a more rapid heart rate than control animals, this was not statistically significant. After an initial marked decrease, SV remained significantly depressed in all dogs throughout the 180 min observation period (Fig. 4). This related primarily to tachycardia that developed in all groups. Naloxone and AVP antagonist groups appeared to have a higher SV than control animals, especially for the first 90 min after treatment, but this was not statistically significant.

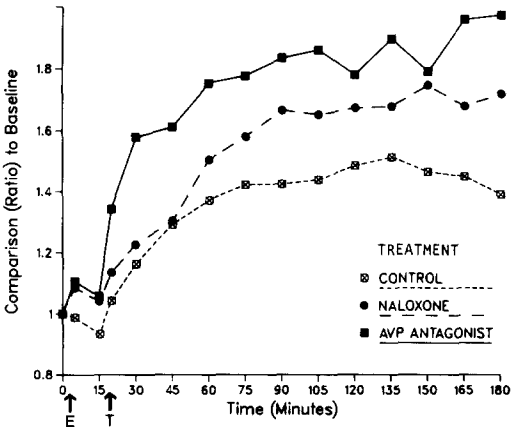

FIG. 3. Tachycardia developed after endotoxin (E) in all treatment (T) groups. AVP antagonist treated dogs developed significantly higher HR than control or naloxone dogs (both $P < 0.05$).

After decreasing to 52% of baseline level immediately after endotoxin administration, MAP remained significantly depressed in control animals for the next 90 min, increasing gradually toward normal thereafter (Fig. 5). Treatment with naloxone or AVP antagonist

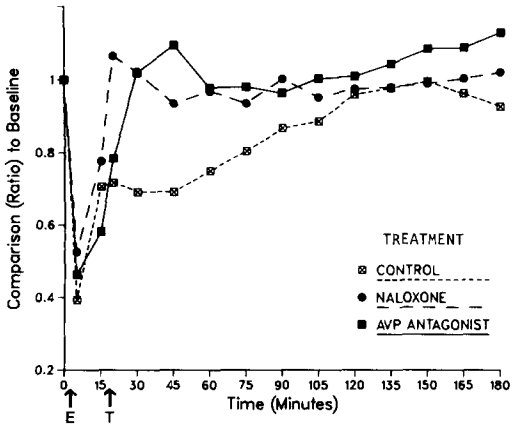

FIG. 2. LV *dP*/dt decreased 50% after endotoxin (E). Compared to control dogs, LV *dP/dt* returned rapidly to normal after drug treatment (T) with naloxone ($P < 0.05$) or AVP antagonist ($P < 0.01$).

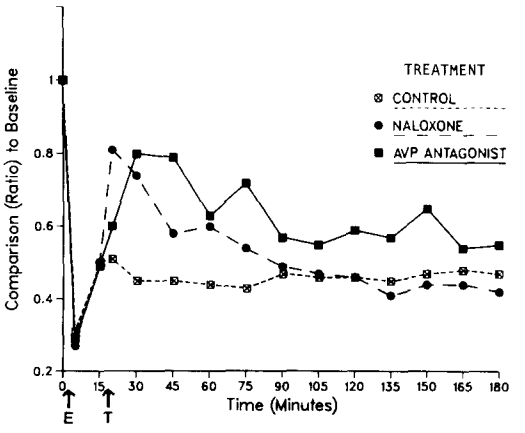

FIG. 4. After endotoxin (E), SV fell markedly and remained depressed. Drug treatment (T) with naloxone and AVP antagonist appeared to initially improve SV, although this was not statistically significant.

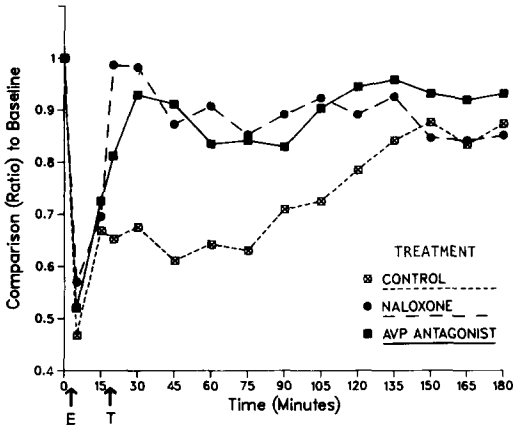

FIG. 5. MAP decreased rapidly after endotoxin (E), returning nearly to baseline level much more rapidly after naloxone ($P < 0.03$) or AVP antagonist ($P < 0.01$) treatment (T) than in control, untreated dogs.

rapidly increased MAP to 90–100% of baseline value. This improvement was statistically different from control animals, where MAP returned to 90% of baseline only after 150 min (naloxone > control, $P < 0.03$; AVP antagonist > control, $P < 0.01$). After endotoxin administration, TPR transiently increased, during the early, transient cardiovascular collapse (Fig. 6). TPR then decreased toward baseline, but remained elevated from 1.2 to 1.5 times baseline in both naloxone and control animals throughout the experiment. Dogs treated with AVP antagonist maintained their TPR below baseline, significantly different than control ($P < 0.04$) or naloxone ($P < 0.02$) groups. Temperature increased progressively throughout the 180 min observation period after endotoxin administration, being statistically comparable in all groups (Fig. 7).

Hematologic variables showed minor, but occasionally statistically significant differences between the three treatment groups at baseline (Table 2). Since treatment groups were randomly assigned, these differences appeared to result from large variations among individual dogs that were not eliminated by the small number of animals in each group. In all cases, these differences were small compared to the subsequent changes observed after experimental manipulation. After endotoxin administration, WBC count decreased markedly (by 30 min) and remained depressed at the 90 min sample time. This decrease was comparable in the three treatment groups, with leukocyte counts decreasing to 15% of their baseline value. Differential counting revealed that the leukopenia was due to a reduction in heterophilic leukocytes (polymorphonuclear forms), which decreased from 85% of the total leukocyte count at baseline to 38% of the leukocyte population 30 min after endotoxin ad-

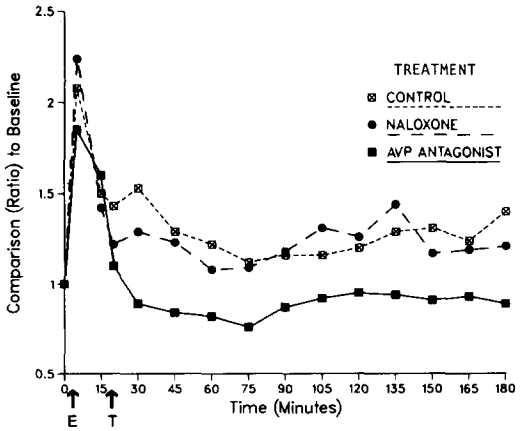

FIG. 6. TPR increased transiently after endotoxin (E), and remained significantly higher in control ($P < 0.04$) and naloxone ($P < 0.02$) dogs, than in dogs treated (T) with AVP antagonist, where TPR was lower than baseline after the initial transient increase.

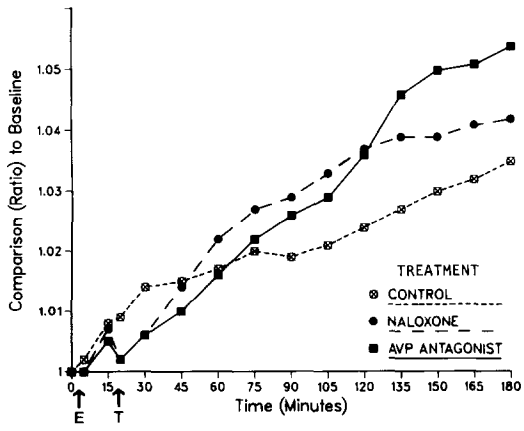

FIG. 7. Temperature increased gradually in all dogs after endotoxin (E), with no significant difference between treatment (T) groups.

TABLE 2

HEMATOLOGIC PARAMETERS

	Treatment group	Baseline	Time 30 min	Time 90 min
WBC (cells/mm^3)	C	14.2 ± 1.9	† 1.3 ± 0.3	† 1.4 ± 0.3
	N	22.5 ± 2.7	† 4.4 ± 0.8	† 2.5 ± 0.5
	V	14.3 ± 1.7	† 1.9 ± 0.6	† 3.0 ± 1.2
PMN (%)	C	84 ± 2	† 28 ± 8	† 26 ± 11
	N	83 ± 4	† 45 ± 11	† 40 ± 8
	V	87 ± 3	† 40 ± 13	† 54 ± 8
Hematocrit (Vol %)	C	39 ± 1	† 44 ± 1	† 50 ± 2
	N	44 ± 2	* 49 ± 3	† 51 ± 2
	V	48 ± 3	† 56 ± 3	† 58 ± 2
Platelets (cells/mm^3)	C	199 ± 17	† 72 ± 11	† 99 ± 15
	N	95 ± 15	† 46 ± 10	† 46 ± 8
	V	135 ± 16	† 49 ± 17	† 53 ± 12
Ionized calcium (mEq/liter)	C	2.53 ± 0.08	† 2.46 ± 0.10	† 2.30 ± 0.13
	N	2.51 ± 0.05	2.48 ± 0.05	2.29 ± 0.11
	V	2.47 ± 0.05	† 2.31 ± 0.05	† 2.24 ± 0.07
Epinephrine (pg/dl)	C	100 ± 23	* 8364 ± 3910	† 4519 ± 1256
	N	263 ± 53	* 6207 ± 2644	† 4986 ± 1891
	V	283 ± 59	* 12332 ± 5031	† 10174 ± 3046
Norepinephrine (pg/dl)	C	133 ± 9	† 1497 ± 519	† 1216 ± 206
	N	224 ± 46	* 1246 ± 608	1818 ± 822
	V	212 ± 36	† 1645 ± 272	† 1803 ± 271
Hemolytic complement (% RBC lysis)	C	64 ± 12	† 26 ± 10	16 ± 8
	N	64 ± 13	* 44 ± 15	40 ± 12
	V	39 ± 14	21 ± 9	23 ± 11
Cortisol (μg/dl)	C	7.8 ± 0.9	† 15.1 ± 2.1	† 16.4 ± 1.3
	N	6.2 ± 1.6	† 12.0 ± 2.8	† 14.2 ± 1.1
	V	11.9 ± 2.2	† 19.7 ± 2.1	† 25.3 ± 2.9
Vasopressin (pg/ml)	C	5.5 ± 0.3	† 140 ± 40	† 58 ± 2
	N	8.5 ± 1.7	† 103 ± 15	68 ± 12
	V	13.2 ± 5.1	† 507 ± 49	† 232 ± 24

Note. $\bar{x}$ ± SEM. Within group statistics (paired Student's t test): * $P < 0.10$, † $P < 0.05$, † $P < 0.01$, comparison of baseline vs 30- and 90-min samples. Between group statistics (non-paired Student's t test): baseline: WBC, N > C,V, $P < 0.04$; hematocrit, V > C, $P < 0.03$; platelets, C > N,V, $P < 0.03$; epinephrine, N,V > C, $P < 0.03$. 30 min: ionized calcium, C,N > V, $P < 0.05$; vasopressin, V > C,N, $P < 0.01$. 90 min: PMN, V > C, $P = 0.06$; cortisol, V > C,N, $P < 0.01$; vasopressin, V > C,N, $P < 0.01$. Abbreviations: C, control, N, naloxone; V, vasopressin antagonist; WBC, white blood cells; PMN, polymorphonuclear leukocytes; RBC, red blood cells.

ministration (Table 2). Lymphocytic and mononuclear cells were preserved. Peripheral platelet count also decreased significantly in all treatment groups after endotoxin administration. After 30 min, platelet counts in all groups had decreased to 39% of baseline, differences that were statistically significant and that persisted for 90 min. Hematocrit increased progressively in all dogs during the study, reaching levels of 123% of baseline

by 90 min after endotoxin administration (Table 2).

During this study, ionized calcium decreased slightly in all dogs, to 91% of baseline by 90 min after endotoxin administration. This decrease was statistically significant only for control and AVP antagonist animals. Dogs treated with AVP antagonist showed a more rapid decrease in ionized calcium, significantly different from control and naloxone animals at 30 min ($P < 0.05$, Table 2). Epinephrine and norepinephrine showed considerable variance between individual dogs, but both catecholamines increased profoundly in all treatment groups by 30 min after endotoxin administration, being statistically equivalent in the three treatment groups (Table 2).

Total hemolytic complement was activated in all treatment groups, as indicated by a decrease in percent lysis of sensitized sheep red blood cells at 30 and 90 min after endotoxin administration (Table 2). There appeared to be more complement activation in control animals than in naloxone or AVP antagonist treated dogs. At 90 min, available hemolytic complement had decreased to 25% of its baseline level in control dogs, compared with decreases to only 63 and 59% of baseline in naloxone- and AVP antagonist-treated dogs. Due to the large variance among all animals, however, these changes were not statistically significant.

Plasma cortisol increased after endotoxin administration in all treatment groups, increasing 1.8 times baseline by 30 min, and to more than twice baseline by 90 min. Although AVP antagonist-treated dogs had significantly greater cortisol levels by 90 min than both naloxone ($P < 0.01$) and control ($P < 0.03$) dogs, the percentage change compared to baseline was comparable in these three groups (increases of 210% in control, 230% in naloxone and 212% in AVP antagonist groups).

Plasma AVP increased markedly after endotoxin administration. By 30 min, AVP in control animals had increased 27 times baseline, with a persistent elevation of 11 times baseline at 90 min. Dogs treated with naloxone showed a 14-fold increase in AVP by 30 min, decreasing to 10 times baseline by 90 min. Although this 30 min AVP value appeared to be less than that seen in control animals, this was not statistically significant (Table 2). AVP antagonist treated dogs showed a much greater (61-fold) increase in plasma AVP at 30 min and a 28-fold increase at 90 min, statistically greater than both control and naloxone-treated dogs ($P < 0.01$). Due to the cross-reactivity of AVP with the AVP antagonist, however, it is estimated that this difference represented the amount of antagonist administered. Assuming an initial volume of distribution for AVP antagonist of 67 ml/kg [22], the 10 μg/kg dose of antagonist would have resulted in a measurable 685 pg/ml AVP activity, based on the 0.46% cross-reactivity. Assuming that the volume of distribution increased and noting the antagonist half-life of 3–4 hr [22], the antagonist activity measured 15 and 75 min after administration is sufficient to explain the difference in AVP activity measured between control or naloxone dogs and the AVP antagonist group.

Although there was no statistically significant difference in survival between treatment groups, there was a tendency for improved survival among AVP antagonist treated dogs (Fig. 8). Mean survival was 1.4 days in control, 2.2 days in naloxone, and 4.0 days in AVP antagonist groups. Median survival of 1, 2, and

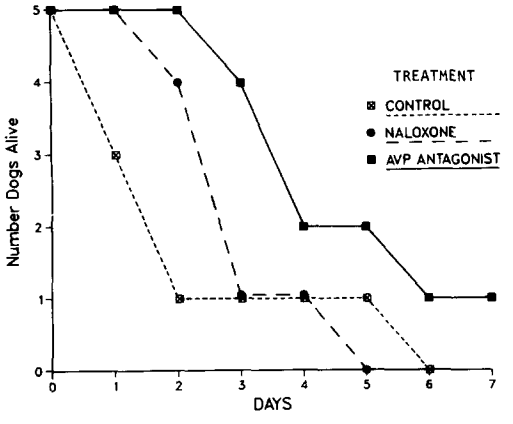

FIG. 8. Survival after *E. coli* endotoxin shock (0.75 mg/kg, LD_{100} at 48 hr) in dogs treated with naloxone, AVP antagonist, or saline (control).

3 days, respectively, for these treatment groups also suggested an improved survival after AVP antagonist treatment, and intermediate survival after naloxone treatment. The only long-term survivor among these dogs was in the AVP antagonist group, and this animal appeared healthy when sacrificed at 7 days. Dogs treated with AVP antagonist were subjectively improved immediately after drug treatment when compared with both control and naloxone dogs. AVP antagonist treated dogs were less lethargic and had fewer symptoms of gastrointestinal emptying than did control or naloxone-treated animals.

DISCUSSION

In this study, *E. coli* endotoxin administered to awake dogs caused a rapid, 25-fold increase in plasma AVP levels. Wilson *et al.* have previously reported increased AVP levels in dogs and baboons after endotoxin or live *E. coli* shock. In their study, the rapid increase in AVP appeared to be stimulated by the effect of decreased venous return on left atrial stretch receptors, since MAP had not yet decreased sufficiently to involve baroreceptors [44]. These investigators found peak AVP concentrations 30 min after endotoxin administration that were equal to or greater than those found in our study. At such high concentrations, AVP is known to have cardiac depressant effects [11]. Even modest doses of AVP reduce CO and increase TPR in normal, conscious dogs [29]. In an isolated canine left ventricle preparation, Wilson *et al.* showed that AVP infusion has direct myocardial depressant effects, decreasing contractility despite relatively normal coronary blood flow [43]. Clinically, myocardial dysfunction with reduced cardiac output is a side effect of AVP infusion used to treat gastrointestinal hemorrhage [38].

The above observations promote the hypothesis that excessively high AVP levels in septic or endotoxin shock might actually have detrimental cardiovascular effects. In this setting, AVP is secreted in response to baroreceptor or left atrial stretch receptor stimuli provoked by hypotension or decreased venous return. These stimuli increase plasma AVP concentration in excess of those normally required to achieve osmotic or fluid balance during water deprivation [44]. Such observations have led to increased recognition of a role for AVP in cardiovascular control [29]. In fact, AVP blockade in dogs subjected to mild hemorrhage results in significantly greater hypotension, suggesting that AVP has a supportive cardiovascular effect during mild hemorrhage. Data from our experiment, however, suggest that in canine endotoxin shock, AVP secretion has gone awry; that is, excessive concentrations of AVP resulted in detrimental cardiovascular effects.

The AVP antagonist used in our study has been shown to predominantly block pressor rather than antidiuretic effects of AVP [22]. It is therefore a useful agent to probe the potential cardiovascular effects of AVP in shock. To our knowledge, this report is the first documentation that AVP blockade after endotoxin administration significantly improves cardiac performance in awake dogs. In fact, AVP antagonist administered 15 min after endotoxin actually normalized CO and MAP by improving LV dP/dt and increasing HR. Tachycardia seen after AVP antagonist may represent an increased cardiac responsiveness to circulating epinephrine, shown to be significantly elevated in these dogs. Archer *et al.* have shown that perfusion of an isolated canine heart with endotoxin significantly reduces the normal cardiac response to epinephrine [1]. Furthermore, Wilson *et al.* have shown that epinephrine can reverse myocardial depression caused by AVP in the isolated canine left ventricle [43].

Since the initial reports of Faden and Holaday, naloxone treatment of endotoxin and septic shock has received considerable experimental and clinical support [7, 8, 31]. In our study, hemodynamic improvements seen after naloxone treatment agree with reports of the salutary effects of opiate antagonism in these studies [35, 36]. Although β-endorphin was not directly measured in our experiment, it would predictably have increased, based on the measured increases in plasma cortisol,

since β-endorphin and ACTH are known to be secreted concomitantly from the same precursor [15, 27]. Experimental evidence of a close interrelationship between β-endorphin and vasopressin secretion has prompted speculation that vasopressin, in fact, might mediate effects attributed to β-endorphin [13]. In rabbits, both IV and third ventricular injection of β-endorphin stimulated a rapid increase in plasma AVP concentration [9, 41]. Naloxone did not block this increased AVP secretion in rabbits, suggesting that a naloxone independent opiate receptor is responsible [9]. In man, however, naloxone has been shown to decrease plasma AVP concentration and prevent AVP increases normally caused by orthostatic hypotension [26]. Thus, data exist to indicate that β-endorphin and naloxone may have different effects on AVP secretion in different species and after different stimuli [14, 20, 21].

Based on the above studies, a complex interaction appears to exist between AVP and β-endorphin. Recent experiments suggest that endogenous opiates may, in fact, promote AVP secretion by inhibiting oxytocin release [2, 39]. Furthermore, Gillies *et al.* reported that AVP may act as a corticotrophin releasing factor in the rat and, therefore, may be capable of promoting endorphin secretion [12]. Based on this potential interaction of AVP and β-endorphin, we were stimulated to measure cortisol and AVP concentration in both naloxone- and AVP antagonist-treated dogs. Our data indicate that the hemodynamic effects seen after naloxone treatment were not mediated by AVP inhibition, since AVP was markedly elevated despite naloxone treatment. Conversely, increased cortisol in dogs receiving AVP antagonist indicates that AVP blockade did not achieve its hemodynamic effects by reducing β-endorphin secretion.

Despite extensive investigation, the underlying mechanisms for cardiac depression and hypotension in endotoxin shock are unclear. Although AVP appears to have a significant role in the current model, its interaction with other vasoactive factors is undoubtedly complex. In an attempt to further characterize our model, a variety of other hematologic parameters were measured. As expected, endotoxin induced marked leukopenia and thrombocytopenia, associated with complement activation. These effects are well documented in this and other endotoxin shock models [30], and were largely unaffected by the drug treatment used in the study. Although not statistically significant, there appeared to be less complement activation and less depression of polymorphonuclear leukocytes in both naloxone- and AVP antagonist-treated dogs. This trend suggests that improved cardiovascular performance might reduce complement activation, which is otherwise aggravated by reduced capillary blood flow.

Reduced ionized calcium in baboon septic shock has been reported by Trunkey *et al.*, who concluded that calcium reduction contributes to decreased myocardial contractility [40]. Although reduced ionized calcium is associated with septic shock, in some cases this reduction might indicate a favorable movement of calcium into cells, essential for the action of certain hormones, such as catecholamines [10]. The early decrease in serum ionized calcium seen in AVP antagonist-treated dogs in our study was associated with positive hemodynamic results, and thus, might reflect favorable cellular calcium uptake and utilization.

Although there appears to be ample support for a direct cardiac mechanism to explain favorable cardiovascular effects produced by AVP blockade in this study, other explanations are possible. In dogs, endotoxin produces marked mesenteric vasoconstriction, as opposed to primates, where slight vasodilation may occur [4]. In fact, sequelae of mesenteric ischemia are a prominant cause of mortality in the canine model. Despite improved early survival of dogs in endotoxin shock after naloxone treatment, significant mesenteric ischemia persists, prompting Reynolds *et al.* to suggest that severe intestinal ischemic damage might abrogate the early beneficial effects of naloxone [36]. High plasma AVP levels found in our study suggest a potential role for AVP in promoting mesenteric vasoconstriction. Relief of this vasoconstriction by AVP block-

ade has the potential to reduce this contribution to mortality in canine endotoxin shock. Furthermore, myocardial depressant factors, suggested by Lefer *et al.* to originate in ischemic intestine during shock [25], might be reduced. If this mechanism proves to be important for the favorable effects of AVP blockade in canine endotoxin shock, considerable caution should be exercised in generalizing these results to primates, where significant splanchnic vasoconstriction does not occur during endotoxin shock.

It would appear teleologically unsound for elevated AVP in endotoxin shock to precipitate myocardial depression. Early cardiovascular collapse during endotoxin shock in rats and dogs, however, is primarily due to hepatic venous pooling that probably stimulates early AVP release [19, 42, 44]. As hepatic venous congestion resolves, this initially favorable AVP response might become excessive. It is known that AVP depresses renin secretion, an alternative pressor mechanism with more beneficial cardiac effects [28]. AVP also reduces cardiac sensitivity to epinephrine [43]. Thus, although AVP may be beneficial in canine or rat endotoxin shock during early splanchnic venous pooling, continuation of this response could be detrimental. If this hypothesis is correct, we would predict that AVP blockade prior to endotoxin administration would have detrimental effects in rats or dogs. In fact, Brattleboro rats that congenitally lack AVP demonstrate significantly worse hypotension after endotoxin administration than Sprague–Dawley rats with AVP present [3]. Additional experiments are required to clarify this hypothesis.

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