Ventricular Fibrillation and Catecholamine Responses during Profound Hypothermia in Dogs

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Profound hypothermia (15 C) was induced in 78 dogs anesthetized with either halothane or ether. Various drugs (propranolol, MJ 1999, P-286, alpha-methyl-tyrosine and metaraminol) were given to inhibit or abolish adrenergic hyperactivity. All dogs except those treated with P-286 had increased levels of circulating catecholamines during cooling. Circulating catecholamines appeared to be more important in the production of ventricular fibrillation than tissue stores. Significant protection against ventricular fibrillation was provided by propranolol and P-286 with ether anesthesia, and by MJ 1999 with halothane anesthesia. Elevation of central venous pressure occurred at 18–20 C and was greatest in dogs treated with beta-adrenergic blocking drugs and P-286. (Key words: Hypothermia; Catecholamines; Ventricular fibrillation; Propranolol; Metaraminol; Alpha-methyltyrosine; MJ 1999; P-286.)

THE USEFULNESS of clinical hypothermia in man is limited by the concomitant development of ventricular fibrillation. Profound hypothermia (15 C) has been attainable only with the Drew technique [1] or modifications,[2, 3] all involving extracorporeal circulation. If intact man could be cooled to 15 C, at which temperature metabolic activity is thought to be virtually nil, advantages undoubtedly would be realized for patients undergoing cardiovascular or neurosurgical procedures.

We hypothesized that the development of cardiac arrhythmias during hypothermia might be related to increased levels of circulating catecholamines. Catecholamine release following acute exposure to cold has been reported.[4, 5] Previous studies from our laboratories indicated that increased catecholamine release accompanied open-heart surgery and hypothermia.[6]

If cardiac arrhythmias during hypothermia are catecholamine-induced, then beta-adrenergic blocking agents such as propranolol (Inderal) or MJ 1999 (4′-[2-isopropylamino-l-hydroxyethyl] methanesulfonanilide hydrochloride) should be beneficial because of their recognized protective effect against catecholamine-mediated arrhythmias.[7, 8] We, therefore, determined whether these blocking compounds (*e.g.*, propranolol and MJ 1999) would prevent ventricular fibrillation during profound hypothermia.

We also explored alternate pharmacologic methods of inhibiting or abolishing catecholamine effects by administering compound P-286, alpha-methyltyrosine, and metaraminol (Aramine). Compound P-286 (N,N-diisopropyl-N′-isoamyl-N′-diethylaminoethylurea) is an experimental drug which allegedly prevents release of catecholamines from the adrenal gland.[9] Bennett *et al.* were able to cool dogs to 11 C with its use. Alpha-methyltyrosine reduces the synthesis of catecholamines by blocking tyrosine hydroxylase.[10] Metaraminol acts as a "false transmitter" and occupies the receptor sites usually occupied by catecholamines.[11]

Methods

We studied 78 healthy mongrel dogs weighing 15 to 20 kg, simulating clinical anesthesia and hypothermia such as might be employed for a neurosurgical operation. Dogs were an-

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TABLE 1. Incidences of Ventricular Fibrillation and Mean Temperature at Which First Episode Occurred

	Number of Dogs	Incidence of Ventricular Fibrillation (Per Cent)	Significance of VF	Mean Temperature (C)
Halothane series				
Group A, control	10	90	—	18.7
Group B, propranolol, 0.2 mg/kg	11	82	NS	18.4
Group C, propranolol, 0.4 mg/kg	6	100	NS	16.4
Group D, MJ 1999, 0.8 mg/kg	12	25	$P < 0.05$	15.6
Ether series				
Group E, control	6	66.6	—	18.8
Group F, propranolol, 0.2 mg/kg	6	0	$P < 0.05$	—
Group G, alpha-methyltyrosine, 50–75 mg/kg	8	62.5	NS	16.6
Group H, metaraminol, 3 mg/kg	7	28.0	NS	18.2
Group I, P-286, 5 mg/kg	6	0	$P < 0.05$	—
Group J, MJ 1999, 0.8 mg/kg	6	16.6	NS	15.5

esthetized with thiopental (Pentothal), 200–250 mg intravenously, and the tracheas were intubated with a cuffed endotracheal tube connected to a circle absorption system. Anesthesia was maintained with nitrous oxide, 1 l/min, oxygen, 1 l/min, and either 0.5 per cent halothane (Fluothane) or approximately 3 per cent ether, vaporized in a Copper Kettle. Ventilation was controlled mechanically at a tidal volume of 20 ml/kg, 12 times/min.

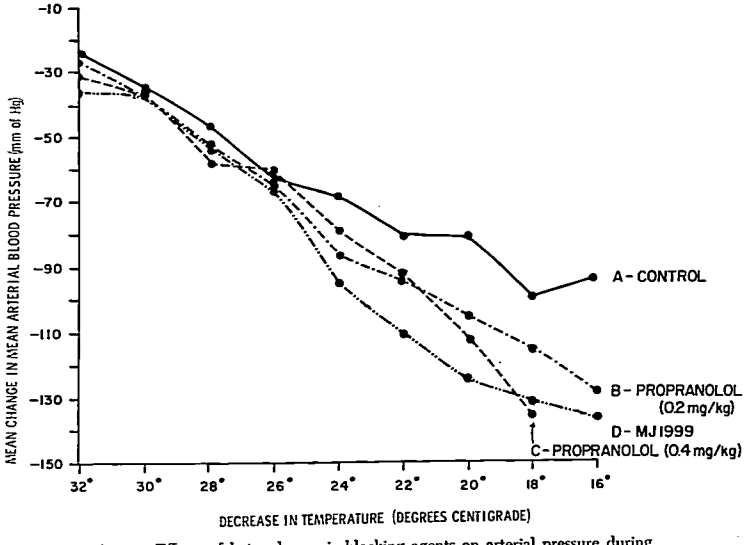

FIG. 1. Effects of beta-adrenergic blocking agents on arterial pressure during hypothermia (halothane anesthesia).

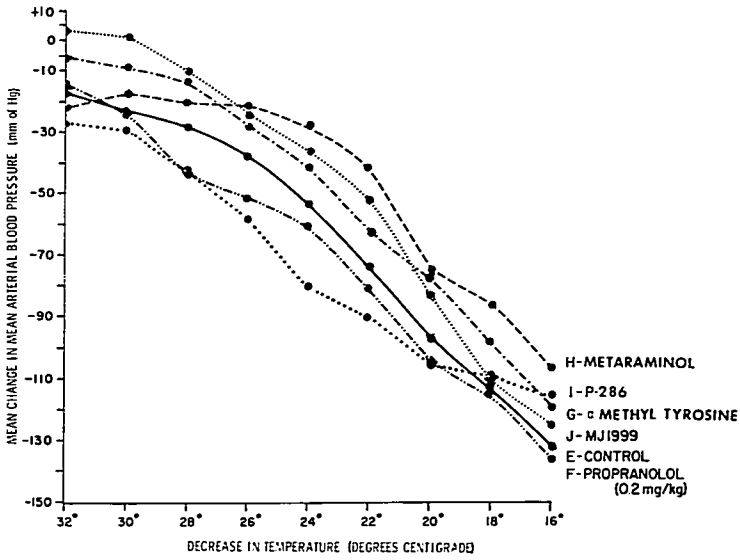

FIG. 2. Effects of beta-adrenergic blocking agents and other compounds on arterial blood pressure during hypothermia (ether anesthesia).

Dogs were cooled with a veno-venous technique in which blood was continuously withdrawn from a femoral vein, circulated through a heat exchanger by means of a Sigmamotor pump, and returned to an external jugular vein. Rate of cooling was kept nearly constant in all animals, averaging 0.5 C/min. Our objective was to cool the animal to 15 C, then rewarm to 30 C. Halothane or ether was discontinued at 20 C, but nitrous oxide and oxygen were continued throughout the experiment.

Femoral arterial pressure, central venous pressure and ECG were monitored continuously on a Gilson recorder via appropriate cannulas and Statham transducers. Esophageal temperature was monitored with a thermistor probe and Yellow Springs thermometer. Mean values of arterial pressure, central venous pressure and heart rate were compared at 2-degree C intervals for all groups of animals. Arterial blood samples were collected prior to cooling, at 15 C, and at 30 C following rewarming. These were analyzed for catecholamines by the method of Anton and Sayre [12] and for blood gases (*p*H, Pa_{CO_2}, Pa_{O_2}) using an Astrup system. A Severinghaus blood gas calculator was used for temperature correction.

An Electrodyne A-C defibrillator was used at an initial setting of 550 volts to defibrillate the hearts, as necessary. Dogs anesthetized with halothane received either propranolol, 0.2–0.4 mg/kg, or MJ 1999, 0.8 mg/kg. Animals anesthetized with ether were given propranolol, 0.2 mg/kg; MJ 1999, 0.8 mg/kg; alpha-methyltyrosine, 50–75 mg/kg; metaraminol, 3 mg/kg; or compound P-286, 5 mg/kg. Propranolol and MJ 1999 were given intravenously 10 minutes prior to cooling. Metaraminol was administered intraperitoneally 12 to 24 hours prior to cooling, and alpha-methyltyrosine was given intravenously 24 hours prior to the experiment in order to achieve catecholamine depletion, as reported by Shore *et al.*[13] and Spector *et al.*[14]

After rewarming to 30 C, the animals were sacrificed with thiopental, 500 mg intravenously. Necropsies were performed in all cases to search for heart worms (*Dirofilaria immitis*) which increased the incidence of ventricular fibrillation in preliminary experiments.

Tissue specimens of both adrenal glands and the apex of the left ventricle were collected and analyzed for catecholamines by the method of Anton and Sayre.[12] Randomization was achieved by withdrawing a protocol-designated slip from a box. For statistical analyses, *t*-test comparisons were used.

Results

The incidence of ventricular fibrillation (VF) in each group of dogs, and the mean temperature at first occurrence, are presented in table 1. Although all dogs given beta-adrenergic blocking drugs fibrillated at lower temperatures than controls, the differences in temperature were not significant. Nine of ten control animals (90 per cent) in the halothane series developed VF at a mean temperature of 18.7 C. Of 11 dogs given propranolol, 0.2 mg/kg, nine (82 per cent) developed VF at a mean temperature of 18.4 C. All six dogs (100 per cent) receiving propranolol, 0.4 mg/kg, developed VF at a mean temperature of 16.4 C. VF occurred in three of 12 dogs given MJ 1999, 0.8 mg/kg, at a mean temperature of 15.6 C. Only the latter group showed demonstrable protection against VF ($P < 0.05$).

Four of six control dogs (66.6 per cent) anesthetized with ether developed VF during cooling at a mean temperature of 18.8 C. None of the six dogs given propranolol, 0.2 mg/kg, fibrillated during cooling to 15 C ($P < 0.05$ compared with controls). Five of eight dogs (62.5 per cent) subjected to ether plus alpha-methyltyrosine developed VF at an average temperature of 16.6 C. Metaraminol-treated dogs had a 28 per cent (two of seven) incidence of VF at a mean temperature of 18.2 C.

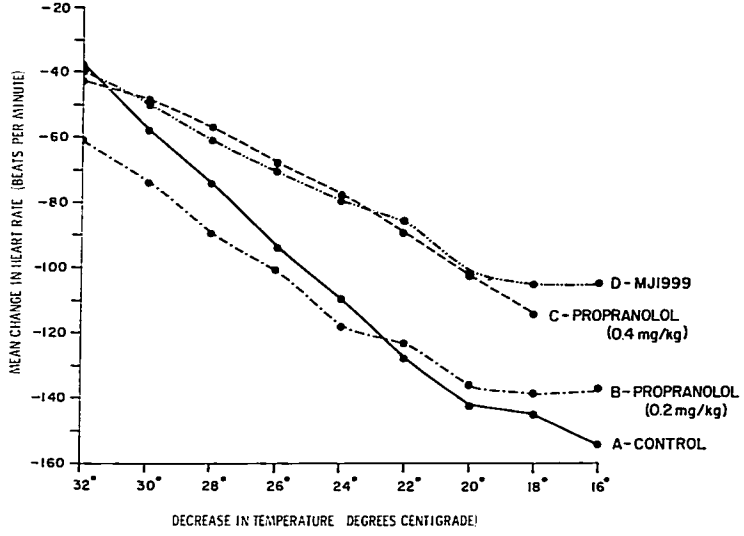

FIG. 3. Effects of beta-adrenergic blocking agents on heart rate during hypothermia (halothane anesthesia).

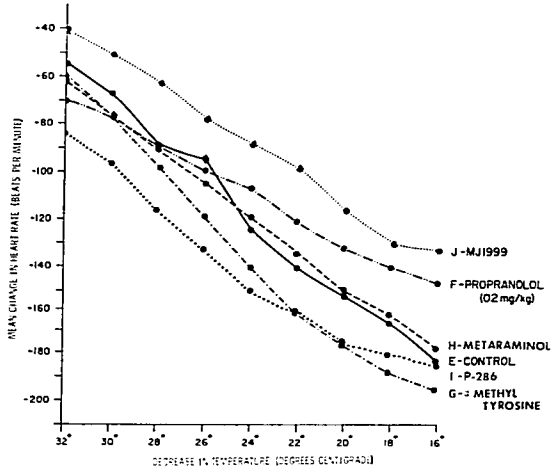

FIG. 4. Effects of beta-adrenergic blocking agents and other compounds on heart rate during hypothermia (ether anesthesia).

None of the dogs given P-286 developed VF. Cardiovascular collapse and death occurred in the first dog given a dose of 15 mg/kg. We gave 10 mg/kg intravenously to a second dog; severe hypotension and bradycardia ensued, but the animal recovered. A dose of 5 mg/kg was given intravenously to the remaining dogs in the group. Only those dogs receiving 5 mg/kg were included in our data. All were cooled to 15 C without fibrillating ($P < 0.05$), confirming the protection against VF during hypothermia reported by Bennett *et al.* In the group receiving MJ 1999, 0.8 mg/kg, only one of the six dogs (16.6 per cent) developed VF, which occurred at a temperature of 15.5 C. This animal had heart worms at necropsy.

Arterial Blood Pressure and Heart Rate

Beta-adrenergic blockade produced no apparent deleterious effects on arterial blood pressure, which fell to 15–25 mm Hg at 15 C. As shown in figures 1 and 2, there were linear decreases in all groups studied. Heart rates also declined linearly to 15 C, where the mean value was in the range of 6–8 beats/min. There did not appear to be significant differences between treated and control animals (figs. 3 and 4).

Central Venous Pressure

All animals maintained stable central venous pressures (CVP) until they reached temperatures of 18–20 C, at which point CVP began to rise (figs. 5 and 6). The increases in CVP occurred earliest in dogs anesthetized with halothane. They were greatest among those receiving beta-adrenergic blocking agents, and significantly higher than controls ($P < 0.01$ for propranolol, 0.2 mg/kg; $P < 0.05$ for propranolol, 0.4 mg/kg, and MJ 1999, 0.8 mg/kg) (table 2).

Dogs anesthetized with ether fared somewhat better, from the standpoint of CVP, than the animals anesthetized with halothane; the increase in CVP occurred at lower temperatures. At 20 C there were no significant increases in CVP among dogs in the ether series, but at 18 C two groups (those receiving MJ 1999 and compound P-286) had significantly higher elevations than controls ($P < 0.05$) (table 2).

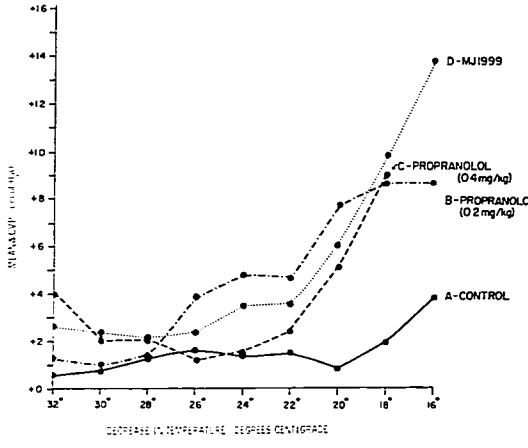

FIG. 5. Effects of beta-adrenergic blocking agents on CVP during hypothermia (halothane anesthesia).

Catecholamines

Plasma catecholamine responses are presented in table 3. All animals except those receiving P-286 showed striking increases in plasma catecholamine levels during cooling. Significance was found only in the group given ether plus propranolol. The small number of dogs in the other groups precluded statistical analysis.

Tissue catecholamine levels are listed in table 4. Dogs treated with metaraminol had significantly decreased catecholamine content in myocardial tissue. All other animals appeared the same as controls with respect to myocardial and adrenal catecholamines. The results suggest that the protection against VF afforded by beta-adrenergic blocking drugs is a result of their acting against circulating catecholamines, not the result of tissue depletion.

Blood-gas Determinations

All animals had moderate respiratory alkalosis during cooling, presumably due to hyperventilation and decreased CO_2 output. We could demonstrate no differences among the groups from the standpoint of *p*H, Pa_{CO_2} or Pa_{O_2}, during either cooling or rewarming.

Discussion

Our results strongly suggest that ventricular fibrillation occurring during hypothermia is catecholamine-induced. Increased levels of plasma catecholamines were demonstrated in all dogs except those treated with compound P-286. We believe this is the result of increased release of catecholamines, not secondary to retarded enzymatic degradation. Hertting *et al.*[15] reported that pharmacologic inhibition of monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) had no effect on circulating catecholamine levels in cats. This suggests that the elevated plasma catecholamine levels observed in our study were not secondary to inhibition of enzymatic activity. Furthermore, other investigators have observed that acute exposure to cold produces increased catecholamine release, as evidenced by urinary excretion studies.[5, 6] Leduc administered catecholamines to rats exposed to cold and concluded there was neither activation of degradation nor inhibition.[5]

Having suggestive evidence that hypothermia causes increased catecholamine release, it seems logical to assume that VF occurring during hypothermia is catecholamine-mediated, and that adrenergic inhibition should protect against this phenomenon. From the stand-

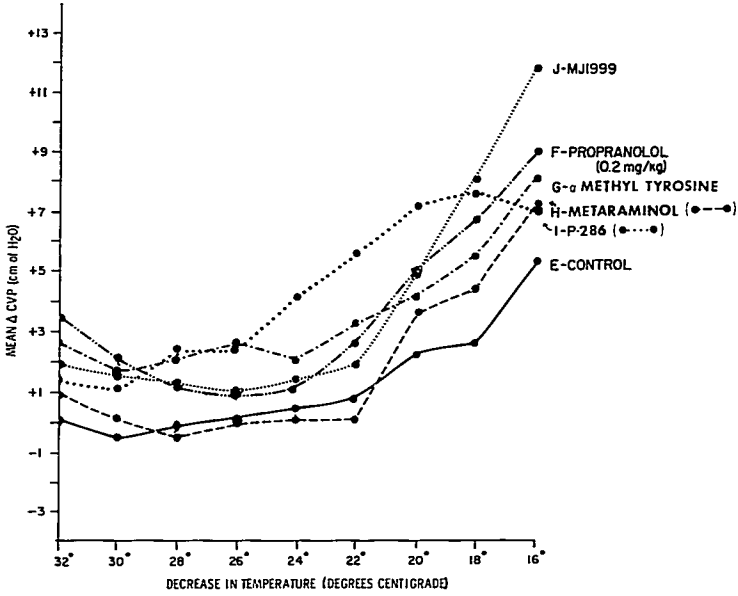

FIG. 6. Effects of beta-adrenergic blocking agents and other compounds on CVP during hypothermia (ether anesthesia).

point of specificity, blockade of beta-adrenergic receptors should provide optimal protection.

On the basis of catecholamine analyses of tissue specimens collected at necropsy, it appears that circulating catecholamines are more important than tissue stores in causing VF during hypothermia. Myocardial catecholamine levels in most dogs did not differ significantly from those of controls. Metaraminol-treated dogs were the exception; these had

TABLE 2. Mean Changes in Central Venous Pressure

	Number of Dogs	Mean CVP (cm/H_2O)	Significance
(Halothane series at 20 C)			
Group A, control	6	+0.83	—
Group B, propranolol, 0.2 mg/kg	8	+7.62	$P < 0.01$
Group C, propranolol, 0.4 mg/kg	6	+6.50	$P < 0.05$
Group D, MJ 1999, 0.8 mg/kg	6	+4.83	$P < 0.05$
(Ether series at 18 C)			
Group E, control	6	+2.50	—
Group F, propranolol, 0.2 mg/kg	6	+6.71	NS
Group G, alpha-methyltyrosine, 50–75 mg/kg	8	+5.50	NS
Group H, metaraminol, 3 mg/kg	7	+4.43	NS
Group I, P-286, 5 mg/kg	6	+7.67	$P < 0.05$
Group J, MJ 1999, 0.8 mg/kg	6	+8.17	$P < 0.05$

TABLE 3. Plasma Catecholamines (μg/100 ml)

	Number of Dogs	Before Cooling			At 15 C		
		NE	E	NE + E	NE	E	NE + E
Halothane series							
Group A, control	4	0.06	0.21	0.27	0.07	0.75	0.82
Group B, propranolol, 0.2 mg/kg	1	0.02	0.17	0.19	0.09	3.81	3.90
Group C, propranolol, 0.4 mg/kg	2	0.01	0.05	0.06	0.01	0.33	0.34
Group D, MJ 1999, 0.8 mg/kg	6	0.02	0.10	0.12	0.07	0.44	0.51
Ether series							
Group E, control	6	0.04	0.16	0.20	0.05	0.23	0.28
Group F, propranolol,* 0.2 mg/kg	8	0.03	0.12	0.15	0.12	0.81	0.93
Group G, Alpha-methyltyrosine, 50–75 mg/kg	6	0.04	0.11	0.15	0.09	0.16	0.25
Group H, metaraminol, 3 mg/kg	5	0.03	0.12	0.15	0.06	0.38	0.44
Group I, P-286, 5 mg/kg	4	0.10	0.10	0.20	0.04	0.09	0.13

* $P < 0.01$.

significant myocardial depletion of catecholamines, yet had no significant protection against VF, presumably because plasma catecholamine levels were almost three times greater than pre-cooling values (table 3). However, necropsy specimens were obtained after the animals had been rewarmed to 30 C and may not have provided an accurate indication of catecholamine levels during profound hypothermia.

TABLE 4. Tissue Catecholamines (Norepinephrine and Epinephrine, μg/g)

	Heart	Adrenals
Halothane series		
Group A, control	0.75 (4)*	688.5 (3)
Group B, propranolol, 0.2 mg/kg	0.90 (1)	
Group C, propranolol, 0.4 mg/kg	0.37 (2)	
Group D, MJ 1999, 0.8 mg/kg	0.57 (8)	
Ether series		
Group E, control	0.47 (6)	1334.5 (2)
Group F, propranolol, 0.2 mg/kg	0.49 (8)	1134.4 (2)
Group G, Alpha-methyltyrosine, 50–75 mg/kg	0.41 (8)	719.2 (1)
Group H, metaraminol, 3 mg/kg	0.13 (7)†	962.3 (2)
Group I, P-286, 5 mg/kg	0.61 (6)	1129.6 (4)
Group J, MJ 1999, 0.8 mg/kg	0.61 (6)	1476.2 (2)

* Numbers in parentheses denote numbers of dogs.
† Significant depletion, $P < 0.01$ (Sheffee criterion).

Propranolol has pharmacologic properties other than beta-adrenergic blockade, e.g., local anesthetic and/or quinidine-like effects.[7] These effects might have contributed to the antiarrhythmic protection observed. It would have been productive to cool an additional group of dogs using a more selective beta blocker. The (−) isomer of propranolol, for example, is 60 to 100 times more potent than the (+) isomer in blocking the inotropic and chronotropic effects of isoproterenol; it also lacks the quinidine-like effect of propranolol and its (+) isomer.[16] MJ 1999, which provided significant protection against VF in the halothane series and also protected five or six dogs in the ether series, also is considered to be a more specific beta-receptor blocking agent.[17] The efficacy of this compound in our experiment suggests that antiarrhythmic effects of propranolol were at least partially the result of beta-receptor blockade.

We cannot explain differences between the results in the halothane and ether series. In the halothane series only MJ 1999 afforded significant protection against VF, while in the ether series both propranolol and P-286 provided significant protection, but MJ 1999 did not. In the latter group, however, only one of six dogs fibrillated (at 15.5 C), and necropsy disclosed heart worms in this animal.

From the standpoint of resistance to VF, ether anesthesia plus beta-adrenergic blocking agents was superior to halothane plus beta-blockers. Twenty-three dogs in the halothane

series were given beta-adrenergic blocking agents (propranolol, 0.2 mg/kg, or MJ 1999, 0.8 mg/kg) prior to cooling, and 12 (52 per cent) fibrillated. Of 12 dogs in the ether series which received the same drugs in identical amounts, only one (8 per cent) developed VF ($P < 0.05$). We could not relate this apparent superiority of ether anesthesia during hypothermia to either plasma or tissue catecholamine levels.

If beta-adrenergic blocking agents are to be utilized in preventing VF during hypothermia, an important consideration is the physiologic cost of this apparent protection. Treated dogs appeared to tolerate cooling without deleterious effects with one exception: rising central venous pressures beginning at 20 C, suggesting impending heart failure. A decrease in venous compliance might also explain the observed elevations in CVP. Adrenergic drugs have been shown to produce venoconstriction.[17] Serum catecholamine depletion, as produced by P-286, theoretically should increase venous compliance. Dogs treated with P-286, the only animals that had decreased catecholamine levels during hypothermia, were second only to those receiving MJ 1999 from the standpoint of elevated CVP (table 2). This suggests that the observed responses of venous pressure were central rather than peripheral in origin. Since we did not measure cardiac output or myocardial contractility, this can only be presumptive. Follow-up studies to assess these factors are planned.

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