Control of Ventricular Fibrillation during Induced Hypothermia in Cats after Differential Depletion of Cardiac Catecholamine Stores with Prenylamine (Segontin)

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ABSTRACT

The role of adrenergic mechanisms for the development of ventricular fibrillation during induced hypothermia was studied in cats. Prenylamine (60 mg/kg) caused a disappearance of the adrenergic transmitter in the muscular nerves; those to the vessels were only slightly affected. Thus pretreated, animals can be cooled to 17.8 to 17°C rectal temperature, and subsequently rewarmed, without developing ventricular fibrillation. Blood pressure was maintained with metaraminol. Without pretreatment the animals constantly develop ventricular fibrillation at 23 to 18.6°C. When prenylamine was given in a dose (20 mg/kg) that does not overtly affect the norepinephrine content of the cardiac adrenergic nerves, ventricular fibrillation was not prevented. In one group of animals receiving 60 mg/kg of prenylamine, norepinephrine instead of metaraminol was infused to maintain the blood pressure during cooling. These animals developed ventricular fibrillation (or cardiac standstill). Fluorescence microscopy showed that the infusion had restored the norepinephrine content in the cardiac nerves previously depleted by prenylamine. The incidence of ventricular fibrillation seems to be related to the number of intact adrenergic nerves present in relation to the cardiac muscles. Hypothermia caused a distinct release of transmitter from the cardiac adrenergic nerves.

ADDITIONAL KEY WORDS

metaraminol
adrenergic mechanisms norepinephrine

Ventricular fibrillation, usually the terminal event preceding death during induced hypothermia in homeothermic animals, is a major risk limiting the clinical use of deep hypothermia. In current investigations on the possible importance of adrenergic mechanisms in the development of hypothermic ventricular fibrillation, attempts are being made to control this complication at different levels, i.e. by interfering with the adrenergic receptors (1, 2), the adrenergic neurons (1), and with the release of catecholamines from the sympatoh-adrenal system to the adrenergic receptors (3).

INPEA (N-isopropyl-p-nitrophenylethanolamine; Selvi) is an adrenergic β-receptor blocking agent, which has an unusual specificity because it has not the local anesthetic, or quinidine-like, side effects common to most other adrenergic β-receptor blocking agents (4, 5). In a previous series of experiments on induced hypothermia in cats (1, 2),...
ventricular fibrillation was controlled by administration of INPEA during the phase of hypothermia (about 20°C body temperature) when the heart appeared to be most susceptible to ventricular fibrillation. Under these conditions the animals could be cooled to 17.9 to 15.2°C rectal temperature and rewarmed to normothermia. After this procedure, they remained in excellent condition during the following week; then they were again subjected to hypothermia, but without INPEA treatment. Ventricular fibrillation then occurred in all at a body temperature of 24.2 to 16.6°C.

In contrast to homeothermic animals, such as cats, which have an adrenergic innervation both of the muscle cells and the vessels of the heart, the hedgehog (1) and the thirteen-lined ground squirrel (Nielsen and Owman, unpublished observations), which are hibernators, were histochemically found to possess little or no adrenergic nerves in relation to the cardiac musculature; on the other hand, the cardiac blood vessels were well innervated by adrenergic nerve fibers. A similar distribution of the nervous catecholamine stores in the heart can be induced by prenylamine (N(3'-phenyl-propyl-(-2')-1, 1-diphenyl-propyl-(3')-amine; Segontin; Hoechst) (6), which causes a partial depletion of the catecholamine stores in the sympathetic-adrenal system (7-12). In view of these findings, cats were pretreated with prenylamine and could then be cooled to 17.8 to 17.0°C rectal temperature and subsequently rewarmed without developing ventricular fibrillation (1); in contrast to this, untreated animals always fibrillated at 22 to 19°C.

EXPERIMENTAL DESIGN

One group of cats was subjected to hypothermia after pretreatment with 60 mg/kg of prenylamine which results in the differential amine depletion of the heart (6) and, judging from previous preliminary findings, should prevent the animals from developing ventricular fibrillation (1). To exclude the possibility that the various effects of prenylamine other than its catecholamine depletion effect (see Discussion) were responsible for its ability to control hypothermic ventricular fibrillation, a second group of animals was given a lower dose (20 mg/kg) of prenylamine which had considerably less influence on the cardiac catecholamine level (6), but which seemed to have side effects in common with the 60 mg/kg dose. These animals might be expected to develop ventricular fibrillation during cooling in the same manner as untreated animals.

During hypothermia it is obviously necessary to maintain perfusion pressure high enough to meet the oxygen requirements of the tissues. Metaraminol (Aramine; Merck, Sharp & Dohme) exerts its hypertensive effects to a large extent by releasing endogenous transmitter from the stores in the adrenergic nerve terminals (13). If a relationship exists between adrenergic mechanisms and hypothermic ventricular fibrillation, metaraminol should further provoke ventricular fibrillation in the case of intact noradrenaline stores and should further potentiate the difference in susceptibility to ventricular fibrillation between those animals in which adrenergic receptors can still be directly influenced by the adrenergic innervation (notably the cardiac muscles) and those "pharmacologically denervated" by prenylamine. Metaraminol was therefore used to maintain blood pressure during cooling of normal animals, and of animals given either 20 or 60 mg/kg of prenylamine. If, on the other hand, the noradrenaline stores of the adrenergic nerves to the cardiac muscles are refilled by exogenous norepinephrine after depletion with prenylamine (60 mg/kg dose), the ability to develop ventricular fibrillation through an adrenergic mechanism should again be restored. Accordingly, a group of animals was pretreated with 60 mg/kg of prenylamine, but the blood pressure was maintained with norepinephrine instead of metaraminol.

The effect of the prenylamine and the influence of hypothermia on the cardiac catecholamine stores was analyzed histochemically, and the results were compared with those from hearts from normothermic control
animals that had received the same prenylamine pretreatment.

Methods

Fifty-five cats of either sex, weighing between 2.2 and 5.6 kg were used. The animals were divided among the different experimental groups so that the mean weights did not differ significantly from each other.

COOLING PROCEDURE

The animals were anesthetized with sodium pentobarbital (30 mg/kg ip) and the trachea was cannulated. Indwelling polyethylene catheters were placed in the left carotid artery and superficial jugular vein. The blood pressure was measured with a mercury manometer and the rectal temperature with a thermocouple inserted about 8 cm into the rectum. Electrocardiograms were obtained from standard limb lead II on an Elema Mingograph at a paper speed of 25 mm/sec. All measurements were continuously observed and the results registered at least every 15 min. Cooling was performed by submersion of the animals in an ice-water bath (about 7°C). Occasionally shivering occurred, usually only at the beginning of the cooling procedure, and then small supplementary doses of pentobarbital were given iv. The animals were allowed to breathe spontaneously; artificial respiration was employed only when indicated: positive air pressure was controlled by connecting a pump respirator with the endotracheal cannula via a T-tube. When the systolic blood pressure fell below 60 mm Hg, intermittent 10% invertose, containing either metaraminol (10 μg/ml) or norepinephrine (Nor-Exadrin cone; Astra, 1 μg/ml), was given iv at a rate of 2 ml/min until the blood pressure again had reached at least 60 mm Hg, after which infusion was stopped. When the pressure subsequently fell below 60 mm Hg, the infusion was again started (see Drug Treatment).

At 18°C rectal temperature, active cooling was interrupted by replacing the ice water with warm water. Rewarming was often initially supported by injection of 10 to 30 ml of warm 0.9% saline (about 40°C) into the arterial catheter. Under these conditions the rectal temperature reversed between 17.9 and 17.0°C. Attempts were then made to rewarms the surviving animals to near normothermia.

DRUG TREATMENT

Group 1a (4 normothermic animals): untreated. Group 1b (10 animals subjected to hypothermia): no pretreatment; blood pressure maintained above 60 mm Hg with metaraminol.

Group 2a (3 normothermic animals): prenylamine 20 mg/kg sc 24 hr before the animals were killed. Group 2b (10 animals subjected to hypothermia): prenylamine 20 mg/kg sc 17 to 26 hr before cooling; blood pressure maintained with metaraminol.

Group 3a (3 normothermic animals): prenylamine 60 mg/kg sc 24 hr before the animals were killed. Group 3b (15 animals subjected to hypothermia): prenylamine 60 mg/kg sc 18 to 24 hr before cooling; blood pressure maintained with metaraminol.

Group 4a: identical with Group 3a. Group 4b (10 animals subjected to hypothermia): prenylamine as in Group 3b; blood pressure maintained with norepinephrine.

HISTOCHEMICAL METHOD

The adrenergic transmitter was directly visualized in the sympathetic nerves of the heart by the fluorescence histochemical methods of Falck and Hillarp (14-17). The hearts from all animals that died as a result of the cooling procedure, the animals rewarmed to normothermia (killed by bleeding), and the normothermic control animals not subjected to cooling (killed by bleeding under pentobarbital anesthesia) were removed, and specimens from the right and left ventricular walls as well as from the atria were quenched in a propane-propylene mixture to the temperature of liquid nitrogen. After freeze-drying, the preparations were treated for 1 hr in formaldehyde gas from paraformaldehyde at 80°C, embedded in paraffin in vacuo, serially sectioned at 6 μ thickness and further treated for fluorescence microscopic analysis of the amines, all according to Falck and Owman (18). Under the conditions used, primary catecholamines, such as norepinephrine, emit an intense green fluorescence. Within certain limits (19) the intensity of the emitted light is a good measure of the relative amount of amine present.

Results

CARDIAC CATECHOLAMINE STORES (ADRENERGIC INNERVATION) IN NORMOTHERMIC ANIMALS

After treatment of the heart preparations in formaldehyde gas for the histochemical demonstration of certain monoamines (14-17), a specific green fluorescence developed almost exclusively in nerve fibers. The characteristics of the fluorophore suggested the presence of a primary catecholamine (18). No fluorescent nerve cell bodies were found (20).

It is generally accepted that the transmitter in the peripheral sympathetic nervous system is norepinephrine (21, 22). Therefore, the green catecholamine fluorescence in the...
FIGURE 1

Hypothermic cat (60 mg/kg of prenylamine; metaraminol). Right atrium. Intensely green fluorescent “chromaffin” cell, issuing a coarse process. The cells are resistant to prenylamine and do not seem to play any significant role in the development of ventricular fibrillation. The picture is composed of two focal planes. 220×.
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nerves seems to represent the stores of—at least mainly—norepinephrine.

Apart from adrenergic nerves, some very few, small groups of intensely green-fluorescent, “chromaffin” cells (23) were occasionally seen in the myocardium (Fig. 1).

Group 1a. The adrenergic innervation of the normal feline heart tissue was organized in principally the same way as that of other mammals (6, 20, 24). The atrial preparations showed a fairly rich distribution of varicose nerve terminals, exhibiting an intense green light owing to their high catecholamine content. Both the muscle cells and the cardiac vessels were enclosed in characteristic autonomic ground-plexa suggesting a true adrenergic innervation of the two components. The terminal fiber system seemed to originate from several thick bundles of mostly smooth preterminal axons with a moderate fluorescence intensity, which entered the heart in the region of the caval veins.

Although less extensive, the adrenergic innervation of the ventricles was arranged in principally the same manner as that of the atria (Fig. 2). The number of nerve terminals was somewhat smaller in the left than in the right ventricle. The nerves reached the ventricles through coarse bundles of preterminals running along the main artery branches at the ventricular surface.
Group 2a. Pretreatment of the animals with the small dose of prenylamine did not overtly reduce the number of adrenergic nerves present in relation to the heart muscle fibers or around the cardiac vessels (Fig. 3). The fluorescence intensity of the adrenergic nerve terminals was sometimes slightly decreased, the reduction being more evident in the muscular than in the vascular nerves. These slight changes were equal in the various portions of the heart analyzed.

Group 3a. The high dose of prenylamine caused marked changes in the cardiac adrenergic innervation (Fig. 4). Thus, very few fluorescent nerves were found to remain in relation to the cardiac muscle tissue of both the atria and the ventricles; the nerves, if present, emitted a weak fluorescence. The fluorescent vascular nerve terminals, on the other hand, were only slightly reduced in number, and the intensity of their fluorescence was moderate to fairly high.

Group 4a. This group is identical with Group 3a.

REACTIONS OF THE ANIMALS TO INDUCED HYPOThERMIA

Results are summarized in Table 1 and in Figures 5 to 8. Immediately upon submersion of the animals into the ice-water bath, the arterial (systolic) blood pressure invariably increased by 10 to 75 mm Hg, even when the anesthesia was deep enough to prevent shivering. During the following cooling procedure, the blood pressure (Figs. 5 to 8) and heart rate gradually decreased in the typical manner accompanying the reduction in body temperature. When the blood pressure approached 60 mm Hg (at mean rectal temperatures of 22.3 to 25.5°C; Table 1), the infusion of metaraminol (in Groups 1b to 3b) or norepinephrine (in Group 4b) was started. Both agents caused, in all four groups, a rapid increase in blood pressure (Table 1). Three animals in Group 1b and 1 animal in Group 2b maintained a blood pressure above 60 mm Hg throughout the cooling procedure, and were therefore not given metaraminol. Artificial respiration was started at about 26°C rectal temperature (Table 1), and the respiratory rate and volume were then kept constant throughout the rest of the cooling procedure.

Group 1b. All animals receiving no prenylamine died of ventricular fibrillation at a rectal temperature of 20.8 ± 0.4°C (mean ± standard error of the mean) as demonstrated in Figure 5. More or less severe ECG changes, including arrhythmia, preceded the ventricular fibrillation in half of the animals, while the
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Group 3b. Recordings as in Figure 5. In the 12 animals surviving the hypothermia without developing ventricular fibrillation, there were few if any, fluorescent nerves to the cardiac muscle fibers; these cats are designated as "denervated," though the nerve fibers to blood vessels were less severely affected. Owing to technical failure, 3 of the animals could not be rewarmed to normothermia (see text). Minimum rectal temperatures achieved: 17.8 to 17°C.

Heart activity in the remainder was regular. The 7 animals receiving metaraminol were given 0.5 to 3.3 mg each during the course of the experiments.

Group 4b. Recordings as in Figure 5. Eight animals developed ventricular fibrillation between 21.5 and 18.1°C rectal temperature. Two animals (*) died in cardiac standstill, preceded by severe ECG changes, at 22.9 and 19.5°C.

Group 2b. All animals in this group (receiving a low prenylamine dose) died of ventricular fibrillation (Fig. 6) at 20.1 ± 0.6°C rectal temperature (Student's t test: not significantly different from Group 1b; P > 0.05). The ratio between the number of animals with severely irregular and regular heart activity was the same as in Group 1b. Nine animals were given metaraminol in doses varying between 0.5 and 2.9 mg.

Group 3b. Of the 15 animals pretreated with a large dose of prenylamine and receiving metaraminol (1.3 to 1.8 mg to each animal) during cooling, only 3 developed ventricular fibrillation (rectal temperature 18.9 ± 0.5°C, which was significantly lower than that in Group 1b, 0.001 < P < 0.01). The remaining 12 animals could be further cooled to lower rectal temperatures (Fig. 7). Rewarming was started at 18°C rectal temperature by replacing the ice water by warm water (31 to 45°C). The rectal temperature of the animals soon reversed, at 17.4 ± 0.1°C (Table 1), which was significantly lower than the lowest mean rectal temperatures measured in the animals from Group 1b, 2b, and

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4b; P < 0.001. Cardiac irregularities occurred in 4 animals during the cooling phase, whereas the remaining 8 animals showed regular heart activity even at the lowest body temperatures. When the rectal temperature fell below 20°C, the animals usually ceased to respond to metaraminol; the blood pressure could therefore not be maintained above 40 mm Hg during the last period of cooling.

As soon as rewarming was started the blood pressure and the heart rate rapidly increased. Only transient cardiac irregularities were registered during the rewarming phase. Two of the animals were given metaraminol only during rewarming, whereas the remaining 10 animals were given metaraminol intermittently during both cooling and rewarming (each animal receiving a total of 0.3 to 3.0 mg). At 24.5 to 30°C the animals began to breathe spontaneously. Nine of the animals were rewarmed to normothermia (Fig. 7). The blood pressure could not be maintained at a satisfactory level in the other 3 animals: Autopsy revealed large quantities of blood in the abdominal cavity of 2 animals (the blood originating from a laceration in the liver accidentally produced at the intra-peritoneal injection of the anesthetic), and the third animal was found to have pneumothorax (probably due to overloading of the lungs during artificial respiration in association with rewarming).

Group 4b. All animals receiving a large dose of prenylamine, but given norepinephrine (0.02 to 0.25 mg to each animal) instead of metaraminol, died before rewarming could be instituted (Fig. 8). Eight animals died as a result of ventricular fibrillation at 20.0 ± 0.4°C rectal temperature (not significantly different from the corresponding temperature of Group 1b; P > 0.05), and 2 animals in cardiac standstill following pronounced electrocardiographic abnormalities (rectal temperatures 22.9 and 19.5°C, respectively). Three of the fibrillating animals had cardiac arrhythmia and other more or less marked irregularities before death. In the remaining 5 animals heart activity was regular.

CARDIAC CATECHOLAMINE STORES (ADRENERGIC INNERVATION) AFTER HYPOTHERMIA

Group 1b. During hypothermia the number and fluorescence intensity of the cardiac adrenergic nerves, both to the muscle fibers and to the blood vessels, showed a slight but distinct reduction (Fig. 9a) compared with the untreated, normothermic animals in Group la. The decrease was evident also in 3 animals that received no metaraminol (Fig. 9b).

Group 2b. Compared with the cardiac adrenergic innervation of the corresponding normothermic animals (Group 2a), the adrenergic nerves of the heart showed in this group the same slight decrease in fluorescence intensity as was observed in the previous group.

Group 3b. The fluorescence microscopic picture of the adrenergic innervation in the surviving animals markedly differed from that registered in the animals developing ventricular fibrillation.

In the 12 animals that survived hypothermia, the number and fluorescence intensity

FIGURE 9 (Top)

(a) Hypothermic cat (no pretreatment, metaraminol, 2 mg). Right ventricle. In comparison with Figure 2 there is a distinct reduction in the intensity of the norepinephrine fluorescence in the nerves after hypothermia. The large number of fluorescent dots in the cardiac muscles represents autofluorescent pigment granules. 100X. (b) Hypothermic cat (no pretreatment, no metaraminol). Right ventricle. The decrease in nerve fluorescence after hypothermia is evident also in those animals receiving no metaraminol. 100X.

FIGURE 10 (Bottom)

(a) Hypothermic cat (prenylamine, 60 mg/kg; metaraminol, 3 mg). Right ventricle. Fluorescent nerves remain only in relation to blood vessels (—>). The arrangement is thus similar to that demonstrated in Figure 4. This "denervated" animal (Fig. 7) did not develop ventricular fibrillation. 100X. (b) Hypothermic cat (prenylamine, 60 mg/kg; metaraminol, 1.8 mg). Right ventricle. A distinct number of fluorescent adrenergic nerves remain both in relation to the cardiac vessels and muscles. This "innervated" animal died in ventricular fibrillation (see text). 100X.

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Hypothermic cat (prenylamine, 60 mg/kg; norepinephrine, 0.15 mg). Right ventricle. Infusion of norepinephrine restores the green fluorescence in the adrenergic nerves, the intensity of which is even higher than that seen in untreated, normothermic animals (Fig. 2). Note that many of the fibers have a conspicuously smooth appearance (see text). 160 X.

The number and fluorescence intensity of the adrenergic nerves did not overtly differ from that of normothermic animals receiving the same 60 mg/kg dose of prenylamine (Group 4a), as is evident from Figure 10a. Thus, a moderate to sometimes fairly high green fluorescence occurred in an almost normal amount of vascular nerves, whereas no (or only very few) adrenergic nerves were found to remain in relation to the heart muscles. The latter nerves, when present, emitted only a faint light.

The number and fluorescence intensity of the adrenergic nerves supplying the cardiac musculature was significantly higher in the 3 animals that died during ventricular fibrillation (Fig. 10b). But the adrenergic innervation to the heart vessels, on the other hand, resembled that found in the 12 surviving animals.

Group 4b. When the large dose of prenylamine was combined with the infusion of norepinephrine, the distribution and intensity of the catecholamine fluorescence in the nerves to the heart differed markedly from that seen after the combined metaraminol infusion (Group 3b). The total number of fluorescent nerves seemed to be the same as in animals that had received either no pretreatment (Group 1b) or a small dose (20 mg/kg) of prenylamine (Group 2b). The fluorescence intensity of the axons was equal to, or even stronger (Fig. 11), than in the untreated, normothermic animals (Group 1a). Moreover, the nerve terminals did not always show the characteristically "beaded" feature, many fibers appeared smooth, like preterminal nerve fibers.

Discussion

The results demonstrate that untreated animals receiving metaraminol during cooling to maintain an arterial blood pressure above 60 mm Hg invariably develop ventricular fibrillation at a rectal temperature of about 20°C. This corroborates previous observations made under similar conditions (2). Pretreatment with prenylamine in a dose not significantly altering the amount or cellular distribution of the cardiac catecholamines (6) does not influence the incidence of ventricular fibrillation. The body temperature at which ventricular fibrillation supervenes seems to be correlated with the amount of norepinephrine present in the adrenergic nerves to the cardiac musculature. When the dose of prenylamine is large enough to produce a severe reduction in the norepinephrine content of the muscular adrenergic axons—with only little influence on the norepinephrine level of the vascular adrenergic nerves (6)—the animals will survive the hypothermia without developing ventricular fibrillation (1). Moreover, the animals can subsequently be rewarmed from 17.0 to 17.8°C.
C rectal temperature to normothermia. When norepinephrine instead of metaraminol is used to maintain a satisfactory blood pressure during the cooling procedure, the adrenergic nerves, notably those to the cardiac muscles, previously depleted of their norepinephrine by prenylamine, can be made to restore the norepinephrine content. Under these conditions even animals pretreated with a large dose of prenylamine develop ventricular fibrillation at the same temperature as those animals receiving no pretreatment.

Prenylamine has a variety of both central and peripheral effects, and therefore several possibilities may be offered to explain why the drug can control the hypothermic ventricular fibrillation. Treatment with prenylamine results in a reduction in the catecholamine content of both the central nervous system and in the sympathetic-adrenal system. Some of the functional changes induced by prenylamine seem to be directly related to this effect. However, prenylamine also produces certain changes in peripheral adrenergic mechanisms that apparently can not be ascribed to its amine depletion effect. It is probable that many of the functional alterations occurring after prenylamine administration are due to more direct effects not necessarily involving adrenergic mechanisms.

It could be possible that some of the last-mentioned kind of actions (e.g. changes in the coronary blood flow, heart dynamics, ECG pattern, carotid sinus reflex [25]) were effective in reducing the incidence of hypothermic ventricular fibrillation. But it seems that such changes can be brought about by even small doses of prenylamine and should therefore be expected to reduce the incidence of ventricular fibrillation at both doses of prenylamine used in the present study. Moreover, the above-mentioned effects are usually only transient.

Prenylamine has been found to possess fairly pronounced quinidine-like effects as seen on the isolated guinea-pig heart (26), which may be due to its local anesthetic properties (25). The mode of administration of prenylamine in the present experiments, however, makes it questionable whether such effects are able to play any significant role in controlling the ventricular fibrillation. This is further supported by the finding that prenylamine did not prevent the development of hypothermic ventricular fibrillation in the presence of norepinephrine.

Prenylamine treatment is known to lower the concentration of brain norepinephrine (7, 8, 10, 11). It is notable, however, that the reduction registered after a subcutaneous injection of 20 mg/kg is not further enhanced by larger doses up to 100 mg/kg (7). Also the brain level of dopamine is lowered (10-12). The course of reduction in brain dopamine at various dose levels closely follows that of norepinephrine, probably indicating an identical mode of action on the stores of the two amines (11). Even large doses of prenylamine have little or no influence on the 5-hydroxytryptamine level of the brain (7, 8, 10-12).

Electrophysiologically, various further changes have been observed in the brain of prenylamine-treated animals (27, 28). If any of these changes were of direct importance in the present experiments, they would probably not have been affected, or reversed, by the infusion of small amounts of norepinephrine; it is well-known that monoamines such as norepinephrine do not pass through the blood-brain barrier (29, 30). It was, however, demonstrated that norepinephrine can antagonize the protective effect of prenylamine on hypothermic ventricular fibrillation.

Against the background of these considerations, it seems reasonable to assume that prenylamine prevents the spontaneous development of ventricular fibrillation during hypothermia by interfering with a peripheral adrenergic mechanism (1-3). It should be noted that prenylamine has a ganglionic blocking effect, which however, seems to be weak and only transient (8). Prenylamine also appears to have a β-adrenergic receptor blocking effect, though this is not quite characteristic (31). It can be observed at a subcutaneous dose of 20 mg/kg, and it was at least antagonized by the infusion of norepi-
nephrine in the present experiment. It seems, therefore, most reasonable that prenylamine interferes with the peripheral adrenergic mechanisms mainly through its catecholamine depletion effect. This is in agreement with the finding that treatment of dogs with reserpine, which markedly decreases the amount of ventricular catecholamine, causes a pronounced reduction in the incidence of ventricular fibrillation upon occlusion of the coronary arteries at 23°C body temperature (32). Coronary occlusion has been shown to produce an enhanced release of norepinephrine from the ventricles (33, 34).

The rise in blood pressure seen after submersion of the animals from all groups in the cooling bath indicates that a sympathetic activation occurred also under the level of anesthesia used. This is further supported by the observations that little or no rise in blood pressure occurs under similar conditions in animals pretreated with bretylium (3). The increase in sympathetic tone during hypothermia or exposure to cold is further reflected by enhanced catecholamine output from the adrenal medulla (35) and augmented utilization of cardiac norepinephrine (36), which probably accounts for the increased levels of blood catecholamines (37).

Although prenylamine lowers the catecholamine content of the adrenal medulla (9, 11), this effect on peripheral adrenergic mechanisms seems to be of minor importance as an explanation of the present results; even adrenalectomy does not prevent the development of ventricular fibrillation during hypothermia (3). Therefore, interest should apparently be focused on the sympathetic nerves. The lower fluorescence intensity of the cardiac adrenergic nerves of those animals subjected to hypothermia, as compared with normothermic animals, strongly indicates a substantial catecholamine release, whether induced by the hypothermia per se or by a combined metaraminol effect (13). It cannot be excluded that the effect of prenylamine in controlling ventricular fibrillation is secondary to its effect on extracardial adrenergic mechanisms. However, the correlation between the norepinephrine content of the adrenergic nerves supplying the heart muscle (but not the heart vessels) and the incidence of hypothermic ventricular fibrillation is striking.

Three of the animals that received 60 mg/kg of prenylamine unexpectedly developed ventricular fibrillation while being cooled. There is strong reason to believe that this can be properly explained by an ineffective reduction of the catecholamine stores, as indicated by the comparatively high fluorescence intensity of the adrenergic nerves to the cardiac musculature. The day after the administration of prenylamine the 12 surviving animals of the same group exhibited characteristic signs of agitation, tremor, ataxia, and muscular weakness (12), as observed before cooling was started, while the 3 animals that later developed ventricular fibrillation appeared slightly sedated but otherwise almost normal. It seems probable that in these latter 3 animals a significant portion of prenylamine has by accident entered the circulation directly during the intended subcutaneous injection. This is supported by the fact that these animals were the only ones that developed severe convulsions shortly after the injection (12, 25). The fluorescence microscopic findings in preparations from their hearts agree well with the more rapid recovery of the norepinephrine levels found after intravenous (12, 38) than after subcutaneous administration of prenylamine (7, 10).

In animals given prenylamine in a dose large enough to deplete adrenergic nerves of their endogenous catecholamine stores, a strong nervous fluorescence could be restored by subsequent infusion of norepinephrine. This indicates that exogenous norepinephrine has been taken up and accumulated in the adrenergic fibers. Since there is good evidence that the granular amine storage mechanism in adrenergic nerves is blocked by prenylamine (39), it could be expected that at least part of the exogenous norepinephrine was present in an extra-granular pool in the axoplasm. This seems to account for the smooth

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appearance of some of the terminal fibers in the heart: The amine was more evenly distributed along the fiber in contrast to normal conditions, where it is accumulated in the varicosities, having the highest concentration of storage granules (40). However, other fibers had a beaded feature with more intensely fluorescent varicosities, indicating that part of the amine was in fact located in the granular storage sites. This is in agreement with the observation (38) that infusion of norepinephrine to animals pretreated with prenylamine will cause a temporary repletion of the amine with a localization in the same subcellular fractions as in untreated animals.

Differential catecholamine depletion from adrenergic nerves by prenylamine (6) can prevent the development of hypothermic ventricular fibrillation (1). This is compatible with the recent findings that specific blockade of the adrenergic /3-receptors (1, 2), or blockade of the catecholamine release from the adrenergic nerve terminals (3) have the same effect. The present experiments lend further support to the hypothesis (1) that adrenergic mechanisms are highly important for the development of spontaneous ventricular fibrillation during induced deep hypothermia.

References
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