Supersensitivity of the chronically denervated feline heart

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It has been shown previously that the extrinsically denervated feline heart manifests a "supersensitivity" to exogenous norepinephrine (NE) (2, 16); however, the mechanism of this augmented responsiveness has not been established. The destruction of intracardiac nerves presumably would make relatively more of an administered dose of agent available at receptor sites, since NE is avidly taken up by intact sympathetic postganglionic axons. Greater availability of NE might thus underlie the greater response of the heart.

Another possible contributory factor might reside in changes in the number or form of receptor sites on the cardiac cell membrane. In order to obtain information on the role that postjunctional receptor site changes might play, we have compared the responses of the denervated heart to NE with the responses of the same hearts to isoproterenol and calcium. Isoproterenol stimulates cardiac beta receptors, but is not believed to be taken up into postganglionic sympathetic nerves as in NE (6, 18). Hence, if the only change in the heart induced by chronic extrinsic denervation is the loss of binding loci for catecholamines which can be taken up by postganglionic sympathetic nerves, then the denervated heart should show no supersensitivity to isoproterenol. If, on the other hand, the denervation is followed by changes in the effector cell, as happens with skeletal muscle (1), then the response to isoproterenol might be expected to be enhanced.

The positive inotropic response to calcium is not blocked by beta receptor blocking agents (14) and consequently is not classified as a beta receptor mediated response. Therefore, the responsiveness to calcium can be used as an index to nonspecific changes in the heart which may have been induced by the chronic extrinsic denervation.

MATERIALS AND METHODS

Adult cats of either sex whose weights ranged from 2.3 kg to 5.0 kg were used in this study. Sixteen cats underwent operation for extrinsic cardiac denervation using the technique of mediastinal neural ablation (3). The operations were performed through a right lateral thoracotomy under halothane anesthesia. The responses of denervated hearts excised between 6 and 23 days postoperatively were compared with the responses of hearts from 20 normal cats.

The animals were anesthetized with pentobarbital sodium (25 mg/kg), following which the hearts were quickly removed and mounted on a cannula. Retrograde perfusion of the coronary arteries was carried out at a constant flow rate (~20 ml/min) with a modified Krebs-Ringer solution (Na+ 146 mM; K+ 3.6 mM; H2PO4 1.2 mM; Ca++ 2.5 mM; Mg++ 1.2 mM; Cl- 128 mM; SO4 1.2 mM; HCO3 25 mM; glucose 5.6 mM; pH 7.4) the temperature of which was regulated (34 C ± 0.5) and oxygenated with 95% O2, 5% CO2.

Mean perfusion pressure varied between 28 and 30 mmHg, and was monitored with a Statham P23Db strain gauge through a sidearm on the perfusion cannula.
Heart rate was maintained at 138/min by electrical pacing at a voltage slightly above threshold in the region of the S-A node. Stimulation was carried out with an American Electronics Laboratory stimulator, model 104A.

The peak pressure developed by the isovolumically beating left ventricle was used as an index of contractility. For this purpose a small Latex rubber balloon mounted on an 18-gauge cannula was introduced into the left ventricle through a small incision in its free wall, and then filled with a small amount of saline. Throughout the experiments the incision in the ventricle was made at the same location. The pressure developed in the balloon was measured by means of a Statham P23Db strain gauge. The pacing artifact, perfusion pressure, and balloon pressure were recorded simultaneously in a direct-writing oscillograph.

Pharmacologic agents were introduced 10 cm proximal to the coronary ostia (volumes of 0.2–0.9 ml) in a manner permitting the injection artifact to become part of the perfusion-pressure record. In each experiment the drug and dose schedules were varied. The drugs used were norepinephrine (levarterenol bitartrate), isoproterenol hydrochloride, calcium chloride, and cocaine hydrochloride. The drugs were diluted in normal saline (adjusted to pH 4.5 with HCl) just as the experiment was begun. Solutions were discarded after each run. It should be noted that injection of a diluent blank in various volumes produced no changes in the base-line parameters. Figure 1 shows the actual tracing from a typical dose-response curve for NE indicating an increasing positive inotropic effect with increasing dose. The balloon pressures at end diastole ranged from 10 to 38 mm Hg. In order to be certain that variations in pressure within the balloon were not important determinants of the magnitude of the responses observed, preliminary experiments were done over a wide range of balloon pressures. Figure 2 shows dose-response relationships measured at three different balloon pressures, indicating no change in the percent of maximum response at three different doses of NE.

Maximum positive inotropic responses of the system were induced in the course of each experiment with calcium or norepinephrine. Greater dose levels of these agents, alone or in combination, as well as high doses of isoproterenol produced only decreases in the magnitude of the maximum inotropic response. The results obtained for all other injections were expressed as percent maximum response. The magnitudes of the responses at each level were compared by paired $t$ testing and the $P$ values indicated. Changes in the median effective dose ($ED_{50}$) between normal and denervated animals were calculated from regression lines for the dose-response curves.

RESULTS

Norepinephrine. In eight out of nine normal hearts there was no increase over base-line in response to $1 \times 10^{-9}$ g NE. All 16 denervated hearts, however, showed a definite positive inotropic response at this dose ($P < .005$). The dose-response curve of the denervated heart lies parallel and to the left of that of the normal heart (Fig. 3). The $ED_{50}$ for NE shifted from $8.5 \times 10^{-9}$ g in the normal to $1.6 \times 10^{-9}$ g in the denervated.

The dose-response relationships for NE were also determined after administration of cocaine ($10^{-6}$ g) to a group of six normal hearts. This dose of cocaine had been previously shown to be maximally effective in the system. There was a shift in the threshold dose as well as a parallel shift of the dose-response curve to the left after cocaine treatment, the magnitude of which was the same as that observed in the denervated group. This is depicted in Fig. 4 in which the dose-response curves both after denervation and after cocaine administration are compared with the normal.

Isoproterenol. The minimum effective doses ($5 \times 10^{-10}$ g) were not significantly different between normal and denervated hearts. Surprisingly, there was a progressive

![Fig. 1. Response of isolated feline heart to increasing doses of norepinephrine as indicated by changes in left ventricular isovolumic pressure on the ordinate. Arrows indicate the point of drug administration. Doses are expressed in grams (g).](http://ajplegacy.physiology.org/)

![Fig. 2. Dose-response relationships for norepinephrine at three different balloon pressures. Ordinate is the percent of maximum response as plotted against dose of norepinephrine in grams on the abscissa.](http://ajplegacy.physiology.org/)
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FIG. 3. Dose-response curves for norepinephrine in the normal vs. denervated feline heart. Ordinate is the percent of maximum response as plotted against dose in grams on the abscissa. P values are: <.005; NS; NS; <.005; NS; <.025; NS, respectively. Bars represent ±1 se.

FIG. 4. Dose-response curves for norepinephrine in denervated hearts and in normal hearts after cocaine administration as plotted in relation to the normal. Ordinate is the percent of maximum response plotted against dose in grams on the abscissa. P values are: NS; NS; <.005; <.05; <.05, respectively. Bars represent ±1 se.

FIG. 5. Dose-response curves for isoproterenol in the normal vs. denervated feline heart. Ordinate is the percent of maximum response plotted against dose in grams on the abscissa. P values are: NS; NS; <.005; <.03; <.05, respectively. Bars represent ±1 se.

FIG. 6. Dose-response relationships to calcium in the normal and denervated feline heart. Ordinate is percent of maximum response plotted against dose in grams on the abscissa. There is no statistical significance between the responses at any dose level. Bars represent ±1 se.

and significant decrease in sensitivity at higher dose levels in the denervated hearts as compared with the normals (Fig. 5). Larger doses of isoproterenol produced arrhythmias in both groups of hearts. The ED50 was 3.7 × 10^-9 for the normal hearts and 6.3 × 10^-9 g for the denervated hearts.

Calcium. The dose-response relationships to calcium were virtually identical in the normal and denervated hearts (Fig. 6).

DISCUSSION

The results of this study confirm the previous observations that extrinsic cardiac denervation results in an augmented responsiveness to exogenous norepinephrine.

With regard to isoproterenol, however, although the threshold remained the same, denervated hearts showed less response than the normals at higher points on the dose-response curve. The reactivities of normal and denervated hearts to calcium were not statistically different.

The implications of these observations are several. First, the magnitude of the shift of the NE curve can be accounted for by the “cocainelike” effect of denervation, that is, a loss of binding sites and thus a greater availability of administered catecholamine for receptor interaction.

Second, since the denervated heart is certainly not more reactive to isoproterenol, it may be at least stated that there does not appear to be a proliferation of post-
junctional sites taking place within the interval of time studied. These data might even lead one to think that there could be fewer beta receptors available in the denervated heart. This view is predicated on the assumptions that isoproterenol exerts its positive inotropic effect only by direct stimulation of beta receptors, and that it and NE stimulate the same receptors.

Third, since various amines may actually be able to "displace" one another in uptake and storage sites (9), one might theorize that some fraction of the net inotropic effect of isoproterenol may be due to local release (or displacement) of NE. Iversen has stressed the fact that there probably is no purely direct-acting or purely indirect-acting catecholamine, but rather that there is a continuous spectrum between these two extremes (10). Realizing that denervation results in a loss of local NE storage pools, one could then view the isoproterenol data as being the result of an "additive effect" of exogenous isoproterenol and endogenous norepinephrine. That is, isoproterenol may release a small amount of extra-neuronal but endogenous NE (4). With increasing doses of isoproterenol the released NE may account for a greater percentage of the total inotropic effect. The difference, then, between the normal and denervated curves (Fig. 5) would represent the percent effect accounted for by NE release in the normal.

Hardman, et al (5) observed that in canine hearts treated with cocaine the contractile force and phosphorylase activity after isoproterenol were significantly less than in the normal. The same preparation showed a supersensitivity of these two parameters to both NE and epinephrine. It could be theorized that cocaine had blocked the local release of NE by isoproterenol and thus resulted in a shift of the curve downward and to the right.

The absence of a change in responsiveness to calcium suggests that the denervation has not induced nonspecific changes in the muscle cell membrane or its receptor sites. The latter distinction is demonstrated in these studies since calcium exerted the same effect in the normal and denervated hearts, whereas the beta-receptor agonists showed modified responses after denervation.

Delineation of mechanisms underlying the responses of the extrinsically denervated heart suffers from the inability to understand completely the terminal apparatus of innervation in the heart and the nature of the neuromuscular junction (11, 13). It is also known that an intrinsic system of cell bodies contained in discrete ganglia and their concomitant axons remain following this procedure. Although it is felt that these represent parasympathetic postganglionic elements, the possibility that some of these intrinsic ganglion cells are synaptically related to the sympathetic nervous system has not been conclusively ruled out.

If in the heart the "ground plexus" is the apparatus through which parasympathetic, sympathetic, and sensory nerve elements are distributed to various sites (7, 8, 11, 13), it would seem that the intrinsic ganglia and nerve fibers would serve to maintain the ground plexus after chronic extrinsic denervation. In contrast to this situation, denervation of skeletal muscle, or postganglionic denervation of smooth muscle by division of virtually all of its postganglionic innervation (as can be accomplished with the nictitating membrane (17, 18) results perhaps in a qualitatively different relationship between the residual of innervation and the effector-cell receptor sites. Prejunctional destruction of innervation is clearly more extensive than can be accomplished by extrinsic cardiac denervation even if this denervation is accomplished by cardiac autotransplantation (15). Therefore, as an extrinsically denervated heart is not a heart without nerves, it is possible that a postjunctional change following denervation has not been documented because there may have been a failure to alter adequately the prejunctional apparatus.

Consequently, these data suggest that the supersensitivity of chronically denervated hearts to NE is based on the loss of intraneuronal binding sites for NE. This conclusion is therefore in agreement with many previous investigators in this area. The techniques used yielded no data which give evidence of a change in the muscle cell membranes or receptor sites in the chronically extrinsically denervated heart.

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REFERENCES


