The Effects of Perhexiline on the Sympathetic Nervous System

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ABSTRACT

The effects of perhexiline on sympathetic nervous system function were studied in vitro and in vivo. Perhexiline decreased the field stimulation-induced contractile response of the isolated vas deferens and significantly decreased the quantity of norepinephrine released during stimulation of this preparation. In vivo studies in dogs showed that perhexiline reduced the heart rate response to cardiac accelerator nerve stimulation, an effect not associated with an increase in cholinergic tone or beta adrenergic blockade. Measurements of norepinephrine released from the heart during cardiac nerve stimulation showed that perhexiline (3 mg/kg) decreased norepinephrine release by approximately 35%. These results suggest that a presynaptic effect of perhexiline, which results in a decrease in norepinephrine release, contributes significantly to the attenuated heart rate response which occurs after administration of this drug. A decrease in transmitter release during sympathetic stimulation could play an important role in the mechanism for the protective effects of perhexiline in myocardial ischemic damage.

Perhexiline, 2(2-dicyclohexyl ethyl) piperidine (Pexid), has been shown to decrease the incidence and intensity of anginal attacks in patients (Bleifer et al., 1972; Morledge, 1973; Hokeenga et al., 1973) and to protect the myocardium from ischemic injury in experimental animals (Daniell et al., 1977). It has been noted that these beneficial effects of perhexiline are associated with a reduction in heart rate response to exercise (Grupp et al., 1970; Pepine et al., 1974a), or the tachycardia commonly observed following acute myocardial infarction (Daniell et al., 1977). It has thus been proposed that the heart rate-limiting effect of this drug and the resultant decrease in myocardial O₂ requirements contribute significantly to the protective effects of perhexiline (Winsor, 1970; Pepine et al., 1973; Daniell et al., 1977). The present study was designed to determine the effects of perhexiline on the sympathetic nervous system to gain further insight into the mechanisms responsible for the chronotropic effects of this drug.

Methods

In vitro studies. Male albino guinea pigs (500–800 g) or male Sprague-Dawley rats (250–300 g) were killed by a blow on the head and exsanguinated. Both vasa deferentia were removed and bathed in Krebs-bicarbonate solution maintained at 37°C and aerated with 95% O₂ and 5% CO₂. The composition of the bath solution was: NaCl, 6.60 g; KCl, 0.35 g; CaCl₂, 0.28 g; K₂HPO₄, 0.16 g; MgSO₄·7H₂O, 0.29 g; NaHCO₃, 2.10 g; and dextrose, 2.08 g; in 1 liter of distilled water. Each vas deferens was placed in a 10 ml tissue bath and arranged between platinum electrodes with the prosthetic end of the tissue tied to a Plexiglas tissue holder and the remaining end attached to an isometric force displacement transducer with a tension of 0.5 to 1 g. The preparation was allowed to equilibrate for 30 min.

For studies of the contractile response of the rat vas deferens, transmural stimulation was performed with a Grass S-4 stimulator with isometric contractions recorded on the Grass model 4 polygraph recorder. Supramaximal stimulation was performed for periods of 15 sec every 4.5 min at a frequency of 10 Hz and a pulse duration of 1 msec. After a control response was established, perhexiline was added directly to the tissue bath and the effects were evaluated.

When the stimulation-induced release of endogenous norepinephrine was studied, the experiments were performed with the guinea-pig vas deferens and proceeded as follows. After the initial 30 min of equilibration, the guinea-pig tissue was intermittently stimulated for 15 sec every 4.5 min as described above. This intermittent stimulation served to stabilize the amount of norepinephrine released per stimulus period. At the end of 30 min the tissue was transferred to a 1.5 ml tissue bath and stimulated (10 Hz, 1 msec duration) continuously for 3 min (S₁ stimulation). The tissue was then returned to a 10 ml tissue bath containing either fresh Krebs-bicarbonate solution or Krebs-bicarbonate solution plus perhexiline. After an additional 45 min of intermittent stimulation, the tissue was again transferred to a 1.5 ml tissue bath and stimulated (10 Hz, 1 msec duration) continuously for 3 min (S₂ stimulation). By comparing the amount of norepinephrine released per stimulus period, it was possible to determine the effects of perhexiline on the stimulation-induced release of neurotransmitter. Whenever norepinephrine release was examined, stimulation was conducted in the presence of desipramine (6 × 10⁻⁷ M) and normetanephrine (1 × 10⁻⁷ M). Norepinephrine outflow samples were immediately acidified with perchloric acid and stored frozen until assayed. Norepinephrine was determined in 50 μl of the acidified bath sample using the method of Henry and co-workers (1976).
To determine the effects of perhexiline on the heart rate response to pre- and postganglionic stimulation, in six dogs the right stellate ganglion was isolated. Platinum electrodes were attached to the right sympathetic trunk just proximal to the ganglion. The nerve was stimulated at 0.3, 1, 3 and 10 Hz for 1 min using a previously determined supramaximal voltage. Heart rate was electronically determined with a tachometer. The electrodes were then repositioned and attached to the stellate cardiac nerve just distal to the ganglion and the stimulation sequence was repeated. After a 30-min recovery period, perhexiline HCl (3 mg/kg i.v.) was administered and following a 15-min stabilization period, the pre- and postganglionic stimulations were repeated. The order of pre- and postganglionic stimulation and the sequence of stimulation intensity were varied among the animals. To assess any influence which cholinergic tone may have had on the effects of perhexiline, in four of these animals, atropine sulfate (1 mg/kg i.v.) was administered and the stimulation repeated for a third time.

To determine the influence which alterations in parasympathetic activity had upon the negative chronotropic effects of perhexiline, in three dogs a bilateral vagotomy was performed before the administration of perhexiline (3 mg/kg i.v.). The rate changes to perhexiline observed in these studies were compared with results obtained in animals receiving perhexiline with the vagi intact.

Beta adrenergic blocking activity of perhexiline was evaluated in three dogs. In these studies heart rate dose-response effects of isoproterenol were determined before and after the administration of perhexiline (3 mg/kg i.v.).

The effects of perhexiline on myocardial norepinephrine release during sympathetic nerve stimulation were studied in eight additional open-chest dogs. In these studies, polyethylene catheters were placed in the aorta near the coronary ostium and in the coronary sinus. These catheters were used to obtain simultaneous blood samples before and during postganglionic cardiac accelerator nerve stimulation (25 V, 5 Hz). The left anterior descending coronary artery was isolated and blood flow through this vessel was determined electromagnetically (Carolina Medical Electronics, King, N.C.). In three of the dogs, norepinephrine release was determined during successive 1-min periods of cardiac nerve stimulation to verify that myocardial norepinephrine release during a second stimulation was equivalent to the first stimulation. In five other dogs, simultaneous aortic and coronary sinus blood samples were taken before and during a control cardiac nerve stimulation and again after the administration of perhexiline (3 mg/kg). Norepinephrine content in these and other blood samples was determined by the method of Henry et al. (1975).

Results

The effects of perhexiline on the field stimulation-induced contractile response of the vas deferens are shown in figure 1. Perhexiline produced a dose-dependent reduction in contractile response with concentrations of $5 \times 10^{-6}$ M reducing the response to 87% of control values and $2 \times 10^{-5}$ M decreasing the response to 26% of predrug values.

The effects of perhexiline on the field stimulation-induced release of endogenous norepinephrine from the guinea-pig vas deferens were studied. The vas deferens were isolated and the stimulation described under "Methods." Under these conditions the initial stimulation period (S1) resulted in an average release of 53.8 ± 4.3 ng of norepinephrine per 100 mg of tissue, and a second stimulation period (S2) resulted in an average release of 55.4 ± 3.9 ng of neurotransmitter per 100 mg of tissue. Thus, the ratio of norepinephrine released on successive stimulations (S2/S1) was 103%. Perhexiline diminished the field stimulation-induced release of norepinephrine in a manner which closely paralleled the effects of this drug on the contractile response. A perhexiline concentration of $2 \times 10^{-5}$ M reduced norepinephrine release with stimulation to 25% of predrug values.

Figure 3 shows the effects of perhexiline on the heart rate response to postganglionic stimulation of the cardiac accelerator nerves. With stimulation, the heart rate increased from basal levels of 120 ± 11 to 205 ± 12 beats/min at 10 Hz. The administration of perhexiline caused a reduction in basal rate to 107 ± 8 and the rate remained approximately 15 beats/min less at each stimulus studied. Analysis of the data using a paired t test showed that the reduction in rate was significant at each of the frequencies studied (P < .05). Similar results were seen with preganglionic stimulation with rate increasing from 118 ± 10 to 205 ± 13 (10 Hz) before drug and from 102 ± 10 to 185 ± 12 beats/min after perhexiline (fig. 4). The administration of
Fig. 3. Effects of perhexiline (3 mg/kg) on the heart rate response to postganglionic stimulation of the cardiac accelerator nerve in open-chest dogs. The nerve was stimulated for 1 min with supramaximal voltage. Perhexiline caused a significant reduction in basal heart rate and the rate achieved with stimulation was less than predrug levels at each of the frequencies studied. * Significant difference from the control response (P < .05; paired t test) (N = 6). Values are means ± S.E.M.

Fig. 4. Effects of perhexiline (3 mg/kg) on the heart rate response to preganglionic stimulation of the cardiac accelerator nerve in open-chest dogs. The nerve was stimulated for 1 min with supramaximal voltage. * Significant difference from the control response (P < .05; paired t test) (N = 6). Values are means ± S.E.M.

Bilateral vagotomy before the administration of perhexiline did not prevent the negative chronotropic actions of this drug. After the vagi were sectioned, perhexiline reduced basal heart rate 17 ± 3 beats/min and this effect was not different from that observed in dogs receiving perhexiline with the vagi intact. Thus, the bradycardia seen after perhexiline appears not to be due to stimulation of vagal afferent receptors (Matsuo et al., 1970).

To investigate the possibility that an action on beta adrenergic receptors might be involved in the chronotropic effects of perhexiline, the dose-response effects of isoproterenol were determined before and after the administration of perhexiline (3 mg/kg i.v.). These results are shown in figure 5. After perhexiline, heart rate changes in response to isoproterenol were clearly not decreased. In contrast, there was a suggestion of an increased response to lower doses of isoproterenol after perhexiline; however, this difference was not significant with the small number of animals studied in this investigation (P < .1).

The effects of perhexiline on arterial and coronary venous norepinephrine concentrations and norepinephrine efflux during electrical stimulation of the cardiac accelerator nerves are shown in table 1. Stimulation of the cardiac accelerator nerves resulted in a slight increase in arterial norepinephrine and a pronounced increase in coronary sinus norepinephrine. After the administration of perhexiline, there was a tendency for coronary sinus norepinephrine concentrations to be lower and norepinephrine efflux to be decreased, but these differences were not significant. However, nerve stimulation after perhexiline resulted in coronary sinus norepinephrine levels which were significantly lower (P < .05) than those observed during the control stimulation. Coronary sinus-arterial norepinephrine differences were reduced approximately 25% when the nerves were stimulated after perhexiline administration. An estimate of total myocardial norepinephrine efflux during nerve stimulation, calculated as the product of arterial and coronary sinus norepinephrine differences times left anterior descending coronary blood flow, is also shown in table 1. Perhexiline significantly reduced the quantity of norepinephrine released during nerve stimulation by approximately 35%. These results are in

atropine did not alter the heart rate response to either pre- or postganglionic cardiac nerve stimulation in the perhexiline-treated dogs. Basal heart rate after perhexiline and atropine was 114 ± 5 and increased with each of the four stimulations to levels which did not differ from those seen prior to atropine.

Fig. 5. Effects of perhexiline (3 mg/kg) on the changes in the heart rate response to isoproterenol in dogs. The rate change to beta adrenergic stimulation after perhexiline was equal to or greater than that seen before perhexiline (N = 3). Values are means ± S.E.M.
contrast to those observed in three untreated animals in which a second neuronal stimulation resulted in norepinephrine efflux from the heart equal to 114% of that seen during the initial stimulation.

### Discussion

Although there is considerable evidence which substantiates the effectiveness of perhexiline in the treatment of angina pectoris (Brown et al., 1976; Gitlin, 1974; Morgans and Rees, 1973; Morledge, 1973; Hitchcock et al., 1977; Burns-Cox et al., 1971; Armstrong, 1977; Winsor, 1970; Alcocer et al., 1973; Bleifer et al., 1972), the precise mechanisms for this drug’s effectiveness remain to be elucidated. On acute administration, perhexiline dilates coronary as well as systemic vessels in animals (Hudak et al., 1970; Cho et al., 1970; Rowe et al., 1970; Daniell et al., 1977) and appears to cause a redistribution of coronary blood flow preferentially to the endocardium (Klassen et al., 1976). In healthy volunteers, perhexiline reduced the heart rate response to exercise (Grupp et al., 1970) and in patients with angina the drug not only delayed the heart rate responses to exercise but also prolonged the time to exercise-induced angina (Pepine et al., 1974a, b; Winsor, 1970; Alcocer et al., 1974). These results have been interpreted to indicate that the observed beneficial effects of perhexiline in myocardial ischemia are at least partially the result of a reduced myocardial oxygen need, secondary to a decreased heart rate response to stress.

It is of interest that perhexiline does not appear to reduce sinus rate after chronic administration (Pepine et al., 1974a; Grupp et al., 1970; Winsor, 1970; Morgans and Rees, 1973); yet, after acute administration to anesthetized animals, a negative chronotropic effect is generally detected (Hudak et al., 1970; Cho et al., 1970; Matsuo et al., 1970; Daniell et al., 1977). In the present study perhexiline reduced both resting heart rate and the heart rate achieved at each intensity of cardiac accelerator nerve stimulation. Although the reason for this differential effect of perhexiline is not apparent, it may be due to the preexisting level of adrenergic tone when the drug is given. Regardless of whether an effect on basal sinus rate is observed, perhexiline treatment consistently reduces the heart rate response to stimulation both in patients and in animals. Such an effect was demonstrated in the present study. Perhexiline reduced the heart rate achieved in response to electrical stimulation of the cardiac accelerator nerves at each of the stimulation frequencies studied.

In the present study an influence on the autonomic nervous system as a possible mechanism for the chronotropic effects of perhexiline was examined in vitro and in vivo. Perhexiline produced a dose-dependent reduction in the field stimulation-induced contraction of isolated vasa deferentia which closely paralleled a reduction in norepinephrine release from this preparation. Furthermore, the studies on the heart rate response and myocardial norepinephrine release to cardiac nerve stimulation in isolated preparations showed that perhexiline not only reduced the rate response over a range of stimulation frequencies but also decreased norepinephrine efflux by approximately 35%. Results of the present study are in agreement with previous reports which indicate that alterations in myocardial cholinergic tone or adrenergic beta blockade appear not to underlie the rate effects of perhexiline (Hudak et al., 1970; Matsuo et al., 1970).

Electrophysiological studies in Purkinje fibers have shown that the actions of perhexiline on automaticity, conductivity and membrane responsiveness appear to resemble those of procaainamide, quinidine and propranolol (Ten Eck and Singer, 1973). Conceivably, a similar membrane effect of perhexiline on neurons may be responsible for the decreased norepinephrine release observed in the present study. Thus, a combination of both pre- and postsynaptic actions may be responsible for the heart rate effects of perhexiline. Moreover, these data imply that a decrease in transmitter release during sympathetic stimulation may represent an important component in the protective effects of perhexiline in myocardial ischemic damage.

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### References


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