Anesthesiology
V 42, No 3, Mar 1975

The Mechanism of the Positive Chronotropic Action of Diethyl Ether on Rat Atria

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Diethyl ether elicited a dose-dependent increase in the intrinsic frequency of contraction of isolated rat atrial preparations. The maximum effect (+34 per cent) occurred with 230 mg ether/100 ml medium. This ether concentration corresponds to a partial pressure of 29.2 mm Hg or 2 MAC. The positive chronotropic action of ether was not reduced in atria obtained from rats pretreated with reserpine (4 mg/kg, ip) although this treatment markedly reduced the effect of tyramine on frequency of contraction. The positive chronotropic response to 0.01 μM isoproterenol was inhibited by the beta-adrenergic antagonist 0.3 μM dl-propranolol but remained unimpaired in the presence of 0.3 μM d-propranolol (a much weaker antagonist). In contrast, the atrial response to ether was similar in the presence of either d- or dl-propranolol. Atropine, in concentrations that completely blocked the negative chronotropic action of acetylcholine, did not increase the frequency of contraction, suggesting that the positive chronotropic effect of ether is not due to an atropine-like activity of ether. Our results indicate that the positive chronotropic effect of ether on isolated rat atrial preparations is not mediated via catecholamine release, nor does it represent direct stimulation of beta-adrenergic receptors or block of cholinergic receptors. (Key words: Anesthetics, volatile, diethyl ether; Heart, atria, diethyl ether.)

ALTHOUGH DIETHYL ETHER is known to have a direct myocardial depressant effect,[1] during clinical anesthesia ether characteristically increases heart rate and cardiac output.[2,3] The maintenance of homeostasis during ether anesthesia is generally attributed to increased sympathetic activity.[4,5] Increased catecholamine levels in blood during ether anesthesia have been reported.[6,7] Arrhythmias during ether anesthesia are infrequent, and when they do occur, are generally of supraventricular origin.[8,9] Ether protects against ventricular arrhythmias during cyclopropane anesthesia.[10–12] If ether-induced tachycardia is primarily catecholamine-dependent, it is intriguing that ether has a protective action against ventricular arrhythmias during cyclopropane anesthesia.

The purpose of the present investigation was to determine whether ether can increase the frequency of contraction of a denervated, *i.e.*, isolated, rat atrial preparation, and, if so, whether it manifests its effect indirectly (catecholamine release from stored granules) or directly (direct beta-adrenergic receptor stimulation). We investigated the chronotropic effects of diethyl ether in rats pretreated with reserpine and in the presence of propranolol.

Materials and Methods

Atrial preparations of male Sprague-Dawley rats, sacrificed by decapitation, were suspended in modified Krebs-Ringer bicarbonate glucose solution at 30 C and *p*H 7.4. The composition (mM) of the medium was: NaCl 120; KCl 4.8; $CaCl_2$ 1.22; $MgSO_4 \cdot 7\ H_2O$ 1.33; KH_2PO_4 1.2; $NaHCO_3$ 25.3; glucose 5.5. The medium was continuously aerated with 95 per cent O_2 and 5 per cent CO_2. A constant resting tension of 750 mg was maintained. Intrinsic frequency of contraction of atria was determined with the aid of a Statham strain gauge and a pen recorder. A stabilization period of 60 min was allowed before readings were taken. Ether was administered by means of an adjustable vaporizer.[13] During ether administration, 1-ml samples of the

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Received from the Departments of Pharmacology and Anesthesiology, Indiana University School of Medicine, Indianapolis, Indiana 46202. Accepted for publication July 19, 1974. Supported in part by USPHS Grant HE 07718, in part with facilities provided by the Heart Research Center Grant H6308 from the National Heart Institute, USPHS, in part by a grant from the Anesthesiology Department of Indiana University, and in part under a Grant-in-Aid from the American Heart Association. Indiana Affiliate, Inc. Presented at the Federation of American Societies for Experimental Biology meetings in Atlantic City, New Jersey, April 1974.

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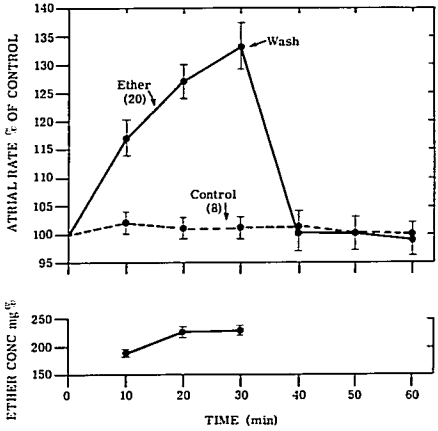

FIG. 1. Effect of diethyl ether on intrinsic frequency of contraction of rat atrial preparations. Atria exposed to approximately 250 mg/100 ml ether for 30 min. Wash represents discontinuation of ether and washout of the preparation three times with medium. Vertical lines represent ± 1 SE. Control represents frequency of contraction of atria in the absence of ether.

medium were taken every 10 min. Ether was extracted by tetrachlorethylene from these samples and concentrations were measured by a gas chromatograph with a thermal conductivity detector.[14] Fresh standards were prepared for each experiment.

The effect of ether on atrial rate was studied in atrial preparations from a) normal rats, b) rats pretreated with reserpine, c) in the presence of 0.3 μM dl-propranolol, and d) in the presence of 0.3 μM d-propranolol.

Reserpine pretreatment of animals was carried out with 4 mg/kg reserpine administered intraperitoneally 24 hours prior to sacrifice. The efficacy of the reserpine pretreatment was tested using tyramine. This method of reserpine pretreatment was found by Kuchii and Shibata to prevent the transient positive inotropic and chronotropic effects of 6-hydroxydopamine in isolated rat atrial preparations.[15]

To study the direct effect of diethyl ether on beta-adrenergic receptors, it was necessary to determine the dose of the beta blocker dl-propranolol that would block the response to an effective dose of a known beta stimulant, isoproterenol. The effective dose of isoproterenol and the dose of dl-propranolol that would block this effect were determined in a separate series of experiments.

Results

Control Intrinsic Atrial Rate and Effect of Diethyl Ether on Intrinsic Rate of Normal Atrial Preparations

In a series of eight experiments, the intrinsic frequency of contraction of the atrial preparation remained stable over 60 min (100 ± 2 per cent, fig. 1). In a series of 20 experiments, diethyl ether (200–250 mg/100 ml) administered over a 30-min period elicited a marked positive chronotropic effect (+33 ± 4 per cent at 30 min). With discontinuation and washout (three times with medium) of diethyl ether, the rate returned to control values (98 ± 3 per cent at 60 min).

Effects of Different Concentrations of Diethyl Ether on Atrial Rate

In a series of experiments following the one-hour stabilization period, the medium was exposed to ether. After a 30-min exposure to ether at a given vaporizer setting, atrial rate and ether concentration in the medium were determined. The data were grouped in 50-mg/100 ml increments of ether concentration and their relation to frequency of contraction noted (fig. 2). The maximal response (+34 ± 4 per cent) was seen with

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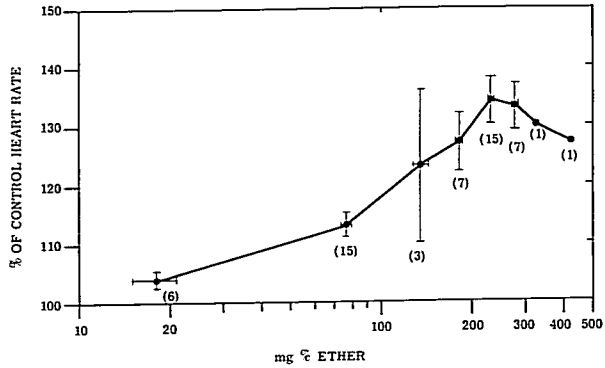

FIG. 2. Ether–atrial rate dose–response curve. Frequencies of contraction of atria were determined 30 min after exposure to diethyl ether. Figures in parentheses represent numbers of experiments in the groups.

ether concentrations of 200–250 mg/100 ml. For further experiments we selected ether concentrations in the range of 150–250 mg/100 ml, representing concentrations close to the peak and in the linear portion of the dose–response curve.

EFFECTS OF DIETHYL ETHER AND TYRAMINE ON RATES OF ATRIAL PREPARATIONS FROM NORMAL AND RESERPINE-PRETREATED RATS

In atrial preparations from normal rats, both tryamine (3×10^{-6} M) and ether (231 ± 9 mg/100 ml) had marked positive chronotropic effects at 30 min (66 ± 9 per cent and 32 ± 3 per cent, respectively (fig. 3). In animals pretreated with reserpine, tyramine had a negligible effect on frequency of contraction ($+9 \pm 4$ per cent), whereas the ether response remained unimpaired ($+50 \pm 4$ per cent, ether concentration 264 ± 4 mg/100 ml). These results indicate that the positive chronotropic effect of ether is not mediated via catecholamine release. In the 28 normal atria represented in figure 3 the frequency of contraction at zero time was 161 ± 3 beats/min. The corresponding rate for atria from rats pretreated with reserpine was 155 ± 8 beats/min. These values were not significantly different ($P < .1$). Thus, reserpine pretreatment did not appreciably alter the intrinsic rate.

DETERMINATION OF AN EFFECTIVE BETA-STIMULANT DOSE OF ISOPROTERENOL

In one experiment, isoproterenol, in increasing concentrations, was added to the muscle bath and its positive chronotropic effect recorded 10 min after its administration. The maximum effect of isoproterenol on frequency of contraction was achieved with 0.03 μM isoproterenol (fig. 4). An almost identical dose–response curve was found in kitten atria by Blinks.[16] For further experiments we selected a dose of 0.01 μM isoproterenol because it, like the ether concentrations we chose, fell near the peak on the linear portion of the dose–response curve.

EFFECTS OF ISOPROTERENOL ON ATRIAL RATE IN THE PRESENCE OF 0.3 μM dl-PROPRANOLOL OR 0.3 μM d-PROPRANOLOL

The positive chronotropic effect of isoproterenol, 0.01 μM, was almost completely blocked by 0.3 μM dl-propranolol (fig. 5), but remained virtually unimpaired in the pres-

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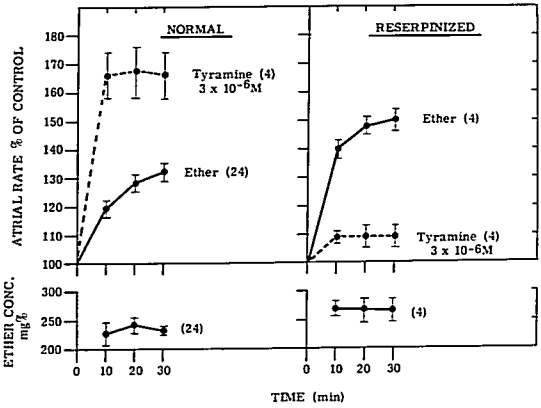

FIG. 3. Effect of diethyl ether or tyramine on intrinsic frequency of contraction of rat atrial preparations from normal rats and from rats pretreated with reserpine.

ence of 0.3 μM d-propranolol. Blinks found that a concentration of 1.0 μM dl-propranolol completely blocked the effects of 0.01 μM isoproterenol in kitten atria.[16] At this and higher concentrations of both dl-and d-propranolol, we found dose-related interference with the intrinsic frequency of contraction of the rat atria. Thus, we chose 0.3 μM dl-propranolol as our blocking dose. At this concentration, blockade was almost complete while minimal effects on intrinsic rate occurred.

EFFECTS OF DIETHYL ETHER ON ATRIAL RATE IN THE PRESENCE OF 0.3 μM dl-PROPRANOLOL OR 0.3 μM d-PROPRANOLOL

In two series of four experiments each, addition of either 0.3 μM dl-propranolol or

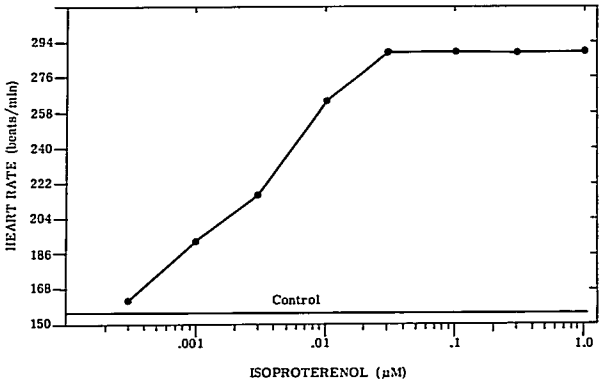

FIG. 4. Isoproterenol–atrial rate dose–response curve.

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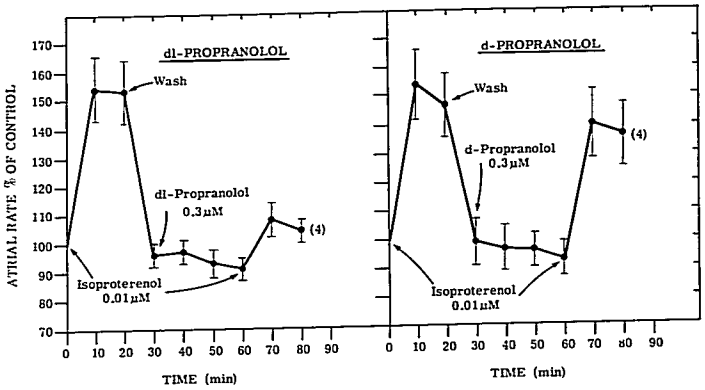

FIG. 5. Effects of isoproterenol on frequency of contraction of rat atrial preparations in the presence of either 0.3 μM dl-propranolol or 0.3 μM d-propranolol.

0.3 μM d-propranolol caused slight depression of frequency of atrial contractions (−1 ± 4 per cent and −5 ± 7 per cent, respectively, at 30 min). Diethyl ether, 150–200 mg/100 ml, elicited a positive chronotropic effect in the presence of either dl-propranolol or d-propranolol (fig. 6). The responses in the two series were almost identical. In both series atrial rate returned to control values after discontinuation of ether and propranolol washout (three times with medium).

EFFECT OF ATROPINE ON ATRIAL RATE

In five separate experiments, acetylcholine (1×10^{-6} M) was added to the bath prior to and following addition of atropine (6×10^{-10}, 6×10^{-9}, 6×10^{-8}, 6×10^{-7} and 6×10^{-6} M). The control frequency of contraction prior to the first addition of acetylcholine was 155 ± 4 beats/min. Acetylcholine depressed the rate to 69 ± 14 beats/min. In concentrations of 6×10^{-10} to 6×10^{-7} M atropine had no effect on frequency of contraction, while at the highest concentration, 6×10^{-6} M, it produced a 7 per cent decrease. The acetylcholine response was unaffected by 6×10^{-10} M atropine, was partially abolished by 6×10^{-9} M, and was completely eliminated by 6×10^{-8} M and all higher concentrations of atropine. Thus, concentrations of atropine that completely blocked the negative chronotropic action of a test dose of acetylcholine did not increase the frequency of contraction of the rat atrial preparation.

Discussion

Our results show that diethyl ether has a direct positive chronotropic action on the isolated atria independent of central innervation. Possible explanations for this action include: 1) a mechanism involving the releasable stores of norepinephrine in the nerve endings and chromaffin tissues of the atria; 2) a direct action of ether on beta-adrenergic receptors in atrial pacemakers; 3) a mechanism not involved with beta-adrenergic receptors.

On the basis of the results of this study, a mechanism involving the release of norepinephrine would appear to be ruled out, since the positive chronotropic effect of ether remained unimpaired in rat atrial preparations pretreated with reserpine, *i.e.*, catecholamine-depleted. The efficacy of reserpine pretreatment was verified by the almost complete abolition of the stimulant action of tyramine. Tyramine is known to elicit its stimulant action via catecholamine release.[17,18]

Our results also eliminate the possibility of

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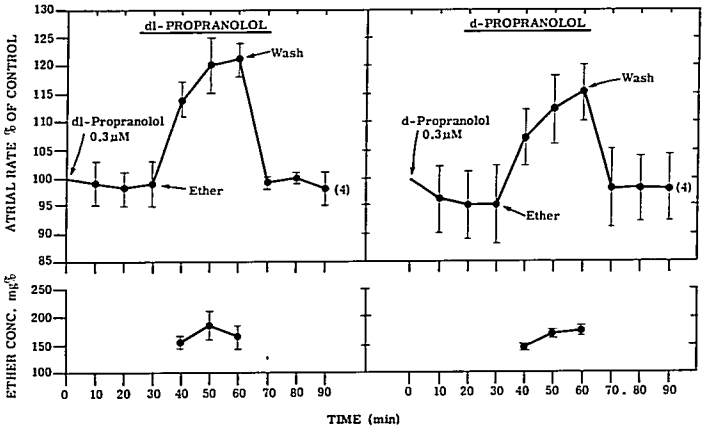

FIG. 6. Effect of diethyl ether on frequency of contraction of rat atrial preparations in the presence of either 0.3 μM dl-propranolol or 0.3 μM-d-propranolol.

a direct effect of diethyl ether on beta-adrenergic receptors. The d-propranolol has all the properties of dl-propranolol except that it is nine times less potent as a beta-blocker than dl-propranolol.[19] Since the responses to ether were almost identical in the presence of dl-propranolol and of d-propranolol, it can be concluded that ether has no direct effect on beta-adrenergic receptors.

Brown and Crout[20] studied the effects of ether on isometric contractility in cat papillary muscles. The presence of dl-propranolol did not alter their findings, and they concluded that diethyl ether does not directly stimulate the beta-adrenergic receptors or the release of catecholamines from adrenergic nerve endings. Our findings are in agreement with their conclusions.

The positive chronotropic action of ether is also unlikely to be due to an atropine-like activity of this anesthetic, since concentrations of atropine that completely blocked the negative chronotropic actions of a test dose of acetylcholine did not increase atrial rate.

We thus conclude that diethyl ether has a direct stimulant effect on rat atrial pacemakers, and that this effect does not involve beta-adrenergic or cholinergic receptors.

Jones *et al.*[2] studied the hemodynamic effects of diethyl ether in man and reported that of all the measured cardiovascular indices only heart rate showed a good correlation with ether concentrations in blood. An analysis of their data indicated that administration of ether for 45 min resulted in a mean arterial blood concentration of 140 mg ether/100 ml and a 35 per cent increase in heart rate. Using the blood–gas partition coefficient of 13.1 at 37 C reported by Eger *et al.*[21] and the Ideal Gas Law, the above blood ether concentration corresponds to 27.82 mm Hg, or 1.91 MAC.[22] Using the water–gas partition coefficient of 20 at 30 C, a partial pressure of 27.82 mm Hg would correspond to a concentration of 218 mg ether /100 ml water (probably almost the same for saline solution). Inspection of figure 2 of our paper reveals a 32 per cent increase in frequency of contraction after 30 min of ether at this concentration.

Thus, if rat and human atria behaved similarly, the effects of ether on human atrial rate could be almost completely explained by the direct action of ether on atrial pacemakers.

Ether is known to increase sympathetic activity[5,6] and depress vagal activity,[24] al-

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though the contribution of these effects to atrial rate in man is not known. Both of these phenomena would facilitate rather than protect against ventricular arrhythmias during cyclopropane anesthesia. Jones *et al.*[9] found serious ventricular arrhythmias with administration of atropine during cyclopropane anesthesia. To our knowledge, the effect of diethyl ether on ventricular pacemakers is not known. A direct stimulant effect of ether on atrial pacemakers might be expected to be effective against some arrhythmias in a fashion similar to atrial pacing.[25] It is conceivable that ether has a more pronounced stimulant effect on atrial pacemakers than on ventricular pacemakers. If the effect of ether on human atrial pacemakers is similar to its effect on rat atrial pacemakers, ether-induced increases in heart rate might explain the protective action of ether against ventricular arrhythmias during cyclopropane anesthesia.

The authors thank Joseph Marovitch and Lance Dell, High School Science Institute students, for technical assistance with parts of this study.

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