Effects of General Anesthetics on Contractile Responses of Rabbit Aortic Strips

Mary L. Price, A.B., and Henry L. Price, M.D.

The functional adequacy of the circulation within tissues can be remarkably independent of changes in perfusion pressure or in total flow. This independence results in part from an ability of the finer blood vessels to respond sensitively to changes in their local environment.

General anesthetic drugs have been found to alter the ability of small blood vessels to respond to normal stimuli in such a way that homeostasis is adversely affected, and to such an extent that survival may be threatened.1-7

These important actions have not been explained. It is by no means certain that they reflect only direct actions of anesthetic drugs upon vascular smooth muscle; there are other possibilities—such as nervous, metabolic, or hormonal reactions to anesthetic drugs—which could mediate or modify these responses. A simple way of distinguishing direct from systemic actions is to study vascular smooth muscle in vitro. This paper describes the direct effects of six general anesthetic agents upon the ability of rabbit aortic strips to respond to the sympathetic nervous mediator, norepinephrine.

Method

Strips of rabbit aorta were prepared and studied in a chamber containing 20 ml. of Kreb’s solution at 37° C., essentially as described by Helmer.1 Modifications of the original method were: (1) animals were killed by injection of air into an ear vein (instead of by concussion and hemorrhage); (2) ethylenediaminetetraacetic acid disodium salt (0.5 mg.) was added to the bath (to reduce oxidation of catecholamines by heavy metals) before addition of test substances, and (3) the aortic strip was suspended in a clear solution consisting of NaCl (22.5 mg.), KCl (2.3 Gm.), anhydrous CaCl₂ (0.92 Gm.), KH₂PO₄ (0.53 Gm.), anhydrous MgSO₄ (0.47 Gm.), NaHCO₃ (7.19 Gm.), and dextrose (6.5 Gm.) in 3.25-liter glass distilled water.

A gas mixture consisting of 5 per cent CO₂ and various percentages of oxygen (range 40 to 95 per cent) was bubbled continuously through the solution in order to maintain pH at 7.5. The remainder of the gas mixture consisted, in various experiments, of nitrous oxide, diethyl ether, cyclopropane, halothane (Fluothane), or chloroform. None of these modified pH. Reduction of the percentage of oxygen in the gas mixture from 95 to 25 volumes per cent (by adding nitrogen) did not affect the contractile response to norepinephrine significantly.

Amounts of thiopental-sodium carbonate mixture* (60 mg. Na₂CO₃/Gm. thiopental) ranging from 0.5 to 2 mg. were added to the bath. These additions increased pH by approximately 0.05 unit. Additions of Na₂CO₃ alone sufficient to increase pH by this amount failed to modify the response of aortic strips to catecholamines.

On the basis of these preliminary experiments we believe the effects to be described represent direct actions of the anesthetic drugs studied, and are not attributable to secondary or indirect influences.

- Norepinephrine bitartrate (Sterling-Winthrop) was freshly prepared for each experiment in a final concentration of 0.03 μg. norepinephrine base/ml. of 0.001 N HCl. The amounts added to the bath ranged from 0.003 to 0.08 μg. In some experiments epinephrine bitartrate (Sterling-Winthrop), 5-hydroxytryptamine creatinine H₂SO₄·H₂O complex (California Fdn. Biochem. Res.), his-

Accepted for publication August 4, 1961. Dr. Price is Wellcome Professor of Anesthesiology, University of Pennsylvania Schools of Medicine, Philadelphia, Pennsylvania.

* As supplied by Abbott Laboratories.
taminy phosphate (Wellcome Labs.), Neosynephrine HCl (Winthrop-Stearns), metham-phetamine (Burroughs Wellcome Co.), or hypertensin (Ciba #59-124) were used to contract the strip. Pitressin and acetylcholine were tried initially, but were found relatively ineffective and erratic in causing contraction.

Responses of the strips were recorded until maxima were attained (7–12 minutes after making additions). Following this the muscle chamber was flushed repeatedly with Kreb's solution which had been stored at 37° C. and through which 5 per cent CO₂ in O₂ had been bubbling. The subsequent response was tested after relaxation was complete (15–20 minutes).

The effect of an anesthetic agent upon the response of the aortic strip was estimated by comparing the contraction elicited by a standard dose of vasoactive substance in the presence of the drug with two “control” (drug absent) observations, one of which preceded and the other of which followed the contraction observed during exposure to the anesthetic. A significant change in the response was recognized only when the contraction elicited during exposure to the anesthetic differed from both control observations by an amount greater than twice the standard deviation of duplicate pairs of control observations. The standard dose of norepinephrine selected was that which produced 2.0 to 3.9 cm. movement of the writing lever tip in the absence of any anesthetic. This is equivalent to 0.15–0.3 cm. contraction, or 5–10 per cent of the muscle length.

Equilibration with the gaseous anesthetic drugs was attained (as evidenced by lack of any further change in the response to norepinephrine) after 10–15 minutes of exposure. Thiopental, which was injected into the bath by means of a syringe, required only 2–4 minutes to produce a maximal effect. For these reasons responses to the amines were measured after 15 minutes exposure to gaseous anesthetics or 5 minutes after thiopental addition. All responses to amines were measured from the level of contraction attained during exposure to the anesthetic alone. When the anesthetic drug itself caused appreciable contraction or relaxation of the strip, the fulcrum of the muscle level was repositioned in order to adjust the recording arm to a horizontal position before adding a vasoactive amine.

Volatile anesthetics were administered by means of calibrated vaporizers; cyclopropane and nitrous oxide from a standard anesthesia machine equipped with flow meters. Anesthetic concentrations were measured as follows: thiopental by the method of Brodie et al., diethyl ether according to Price and Price, cyclopropane by the method of Linde and Price, and nitrous oxide by subtracting the oxygen concentration measured with a Beckman analyzer.

In some experiments the quantity of norepinephrine remaining in the bath at various times after its addition was estimated by the method of Price and Price.

Results

EFFECTS OF ANESTHETIC AGENTS ALONE: Cyclopropane, thiopental, and chloroform commonly caused contraction of the muscle equivalent to 5–15 per cent of the response produced by the standard doses of norepinephrine (ranging from 0.01 to 0.08 μg.). Diethyl ether had no definite effect. Halothane sometimes caused relaxation, but because of the stretched condition of the muscle, relaxation was difficult to elicit and this effect was inconsistent. Indeed, contraction of the strip was also sometimes observed upon adding halothane.

EFFECT OF ANESTHETIC AGENTS ON AORTIC STRIP RESPONSES TO NOREPINEPHRINE: These results are summarized in table 1, where peak responses are compared in the presence and absence of anesthetic agents. The effect of cyclopropane is shown graphically in figure 1.

Cyclopropane, chloroform, and thiopental were most potent in increasing the amounts of contraction caused by norepinephrine. Nitrous oxide had a weaker, although definite, action in the two aortic strips tested. Diethyl ether had no consistent effect. Halothane was unique among the agents studied in reducing the response to norepinephrine, often to as little as half the control response.

MODE OF ACTION OF CYCLOPROpane IN INCREASING RESPONSES TO NOREPINEPHRINE:
Four possibilities were considered, namely, that cyclopropane acted: (1) by reducing the rate of norepinephrine metabolism and/or oxidation in the muscle chamber; (2) by increasing the rate of access of norepinephrine to its site(s) of action; (3) by "sensitizing" the tissue receptors for norepinephrine, or (4) by affecting the contractile process independently of any action on receptors.

The first possibility is unlikely for several reasons. Two series of analyses of the bath fluid made ten minutes after addition of norepinephrine did reveal a slightly (16 per cent) greater concentration of the amine in the presence of cyclopropane than in its absence, but the difference in concentration was within the error of the analytical method and, even if real, was too small to account for the degree of potentiation caused by cyclopropane (cf. table 1). Moreover, both Neo-synephrine and methamphetamine, which are slowly metabolized in comparison with norepinephrine, nevertheless produced a much greater response in the presence of cyclopropane than in its absence (table 2).

The second possibility is also unlikely because, when cyclopropane was added to the bath after the nortie strip had responded fully to a standard dose of norepinephrine, a further marked contraction occurred. The contraction caused by cyclopropane in the presence of added norepinephrine was more than five times as great as that caused by cyclopropane in the absence of a norepinephrine addition.

The third possibility—that cyclopropane acted by sensitizing specific receptors—is also unlikely. Three substances (histamine, hydantoin, and 5-hydroxtryptamine) none of which is a sympathomimetic amine, and none of which acts importantly on the receptors which are specifically sensitive to sympathomimetic amines, nevertheless caused increased contraction in the presence of cyclopropane. The same was true of methamphetamine which, since it lacks catechol structure, is thought to affect contraction by indirect actions which do not involve combination with adrenergic motor receptors. Table 2 summarizes these findings.

The foregoing data favor—by process of elimination—the possibility that cyclopropane increases the response of aortic strips to norepinephrine by affecting the contractile process itself. The linear nature of the response (cf. fig. 1) makes it entirely possible that increased contraction in the presence of cyclopropane represents nothing more than superposition of independent contractile responses to cyclopropane and norepinephrine.

### Table 1. Effect of Various General Anesthetic Agents on Contractile Response to Norepinephrine

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration</th>
<th>Number</th>
<th>Response (Per Cent of Control)</th>
<th>Ob.</th>
<th>Strips</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclopropane</td>
<td>9–16 vol.%</td>
<td>4</td>
<td>133</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30–45 vol.%</td>
<td>2</td>
<td>170</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45–50 vol.%</td>
<td>6</td>
<td>188</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrous Oxide</td>
<td>50–60 vol.%</td>
<td>2</td>
<td>123</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiopental</td>
<td>2.5 mg.</td>
<td>2</td>
<td>140</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.5–8.8 mg.</td>
<td>2</td>
<td>172</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diethyl Ether</td>
<td>90 mg.</td>
<td>2</td>
<td>103</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>120–180 mg.</td>
<td>2</td>
<td>107</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>1–2 vol.%</td>
<td>3</td>
<td>160</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Halothane</td>
<td>1 vol.%</td>
<td>7</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Discussion

These results raise a number of interesting points in the interpretation of previous publications. First, the ability of cyclopropane (when given systemically to dogs) to increase the responsiveness of omental vascular smooth muscle to epinephrine reported by Hershey and co-workers need not be ascribed to a systemic response to these drugs. It is equally possible that increased sensitivity to epineph-

### Table 2. Effect of Cyclopropane (30–40 Per Cent) on Contractile Response to Various Substances

<table>
<thead>
<tr>
<th>Substance</th>
<th>Number</th>
<th>Response During CAFs (Per Cent of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensin</td>
<td>4</td>
<td>215</td>
</tr>
<tr>
<td>Histamine</td>
<td>1</td>
<td>200</td>
</tr>
<tr>
<td>5-HT</td>
<td>2</td>
<td>120</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>1</td>
<td>210</td>
</tr>
<tr>
<td>Neo-synephrine</td>
<td>1</td>
<td>180</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>1</td>
<td>140</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>2</td>
<td>170</td>
</tr>
</tbody>
</table>
rine resulted from direct local actions of the anesthetic similar to those observed in the present study. This possibility is supported by the fact that diethyl ether, which the previous authors found not to increase responses to epinephrine, was similarly ineffective in the present study. However, in the dog, increased responsiveness of the omental vessels occurred as the depth of anesthesia was increased only during the administration of cyclopropane. With both diethyl ether and thiopental, reactivity was reduced with increasing depth of anesthesia and marked vasodilatation occurred, with slowing of blood flow through the capillary bed. Even during cyclopropane administration responses to epinephrine became stronger only in the transition from “light” to “moderate” depth of anesthesia; further deepening caused epinephrine reactivity to diminish again toward the values observed in “light” anesthesia. In our studies, responses to norepinephrine became greater as concentration of cyclopropane or thiopental was increased, while in the case of diethyl ether there was no effect, irrespective of dose, up to concentrations of 200 mg. per cent. This suggests that local actions of the anesthetics on the blood vessels studied by Hershey et al., may not explain all of their observations.

In another study, Burn showed that intense vasoconstriction caused by intra-arterial injections of thiopental in normal animals could be prevented if the tissue stores of norepinephrine were first depleted by pretreatment of experimental animals with reserpine. This led him to suggest that thiopental caused release of norepinephrine from the untreated muscle, with consequent vasocostruction. Unfortunately, he did not substantiate his thesis by showing that thiopental actually did release norepinephrine from the tissues. We believe that an increased response of vascular smooth muscle to existing levels of sympathetic nervous “tone” or activity could also explain his results. The importance of determining the mode of action involved is obvious, if for no other reason than that rational therapy of this catastrophe in man requires such knowledge. If Burn is correct, marked tissue damage may already have been done as soon as norepinephrine is released, and attempts to antagonize its action (e.g., by intra-arterial injections of phenolamine) could fail because of inability of the drugs employed to reach the ischemic tissues. The other possibility—vasoconstriction caused by increased responsiveness to sympathetic nervous activity could be antagonized by blocking sympathetic pathways supplying the affected part.

The ability of certain anesthetic drugs to modify responses to the sympathetic mediator makes it conceivable that changes in vascular tone which occur during their administration result in part from local actions on vascular smooth muscle. The relatively pronounced visceral vasoconstriction reported during cyclopropane administration, and increased total peripheral resistance during thiopental administration may in part represent enhanced responses at normal levels of sympathetic nervous activity. Conversely, vasodilatation induced by halothane may be attributed in part to actions which render vascular smooth muscle relatively unresponsive to norepinephrine and, presumably, also to sympathetic nervous activity.
Although the discussion has dealt at length only with responses to norepinephrine, it seems likely, in view of the apparently unspecific character of the changes induced, that increased vascular tone, irrespective of its cause, could be further augmented by cyclopropane and by other anesthetics which share its actions on smooth muscle contractile processes.

Summary and Conclusions
The actions of six general anesthetic drugs upon the contractile response of rabbit aortic strips was tested. Responses to standard doses of norepinephrine were augmented by cyclopropane, thiopental, nitrous oxide, and chloroform. Diethyl ether exerted no obvious effect. Halothane severely depressed the response. The mechanism responsible for the action of cyclopropane was investigated and found to result most probably from a reversible modification of the contractile process itself.

Supported (in part) by a grant (H1568 C6) from the National Institutes of Health.

References