Laboratory Report

Effects of Cyclopropane on Catecholamine Release from Bovine Adrenal Medulla

Koji Sumikawa, M.D.,* Yoshikuni Amakata, M.D.,† Takeshi Kashimoto, M.D.,‡ Futoshi Izumi, M.D.§

The direct effects of cyclopropane on both adrenal medullary release of catecholamines and release of catecholamines from isolated chromaffin granules were examined. Cyclopropane had no effect on the spontaneous release of catecholamines from perfused bovine adrenals, while it reversibly inhibited carbachol-induced release of catecholamines in a dose-dependent manner. At 20 per cent cyclopropane, catecholamine release was reduced to 52 per cent of control. Cyclopropane exerted no action on spontaneous release or Mg^{++}-ATP-dependent release of catecholamines from isolated chromaffin granules. Thus, the authors conclude that the direct action of cyclopropane on the adrenal medullary release of catecholamines is inhibitory, and that chromaffin granules are not the site of action of this anesthetic. Key words: Anesthetics, gases: cyclopropane. Sympathetic nervous system: anesthesia; catecholamines.

DECREASED PLASMA CATECHOLAMINE LEVELS during halothane anesthesia have been ascribed to suppression of the sympathoadrenal system.[1] The mechanism appears to be central in part,[2] but mainly involves reduction of catecholamine release from the adrenal medulla by a direct peripheral action.[1,3] On the other hand, cyclopropane has been reported to elevate plasma catecholamine levels.[4–6] The elevation of plasma catecholamine levels induced by cyclopropane has been thought to be due to stimulation of the sympathoadrenal system[4,5]; however, it has been shown recently that cyclopropane has a depressant action on the central sympathetic nervous system[7] and an inhibitory effect on the release of norepinephrine from sympathetic nerve endings.[8]

The purpose of this study was twofold: 1) to clarify

the direct effect of cyclopropane on catecholamine release from the adrenal medulla, since the greater part of plasma catecholamines originates from this source,[1] and 2) to ascertain the effect of cyclopropane on the chromaffin granules, since an effect on the catecholamine storage granules has been hypothesized in relation to the site of the action of this anesthetic.[8,9]

Materials and Methods

Fresh bovine adrenal glands were used throughout. The details of procedure were described previously.[10,11]

PERFUSION OF ADRENALS IN VITRO

The glands were perfused via the adrenal vein at a rate of 5 ml/min with a warmed (37 C) modified Locke solution (in mM: NaCl 154, KCl 5.6, $CaCl_2$ 2.2, glucose 10, Tris–HCl buffer 40; pH 7.4), aerated with 95 per cent O_2 and 5 per cent CO_2. About 45 min were allowed before any treatment in order to achieve equilibrium. The adrenals were stimulated twice or more with 0.1 mM carbachol, which was added to the Locke solution. The stimulation period was 6 min, followed by 15- or 30-min recovery intervals. Cyclopropane or nitrogen was added to the aeration mixture during a period starting 15 min prior to the second stimulation and lasting until the end of the second stimulation. The third stimulation was done in the absence of cyclopropane and nitrogen. The effluent from the adrenal was collected at 2-min intervals and the catecholamine content of each fraction was measured fluorometrically.[12] Cyclopropane did not interfere with the measurement. Carbachol-induced catecholamine release was calculated as the difference between spontaneous catecholamine release and release during stimulation.

RELEASE OF CATECHOLAMINES FROM GRANULES

Adrenal medullary granules were isolated by the millipore filter technique[13] and suspended with 150

* Assistant Professor, Department of Anesthesiology, Shiga University of Medical Science.

† Professor, Department of Anesthesiology, Shiga University of Medical Science.

‡ Assistant Professor, Department of Pharmacology, University of Occupational and Environmental Health.

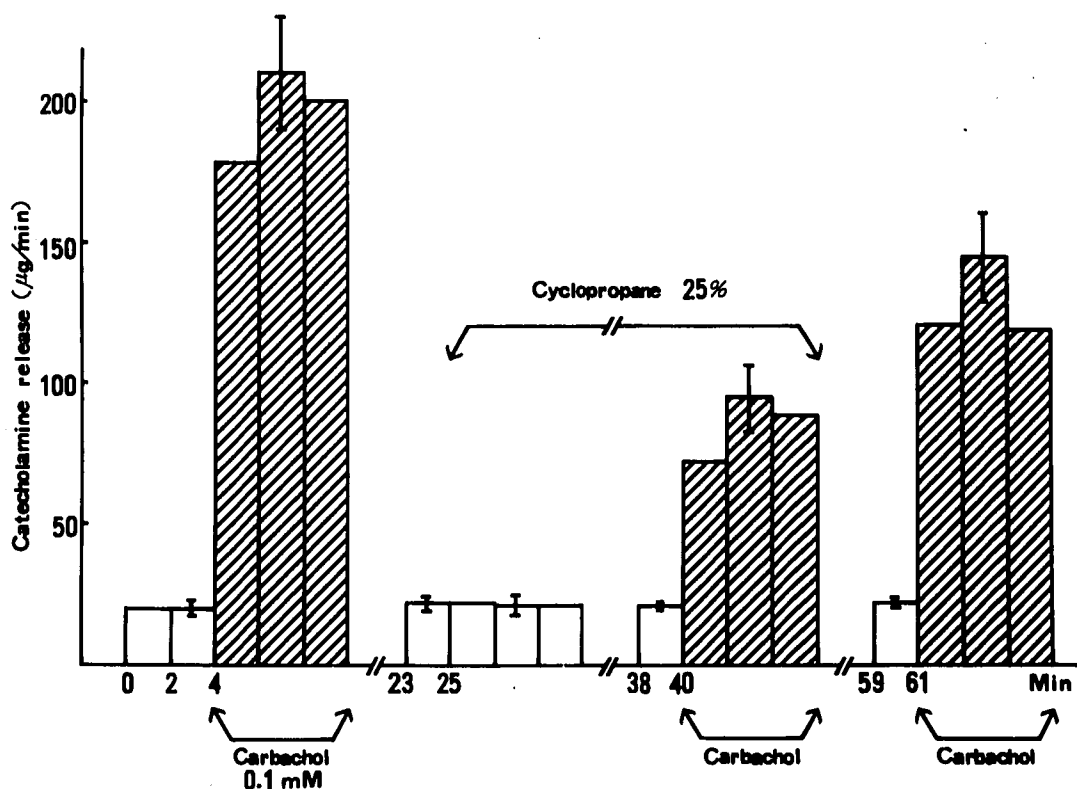§ Professor, Department of Pharmacology, University of Occupational and Environmental Health.

FIG. 1. Effects of cyclopropane on spontaneous and carbachol-induced catecholamine release from perfused adrenals (n = 3, means ± SE). The ordinate represents the rate of catecholamine release during a 2-min period. Adrenals were stimulated three times with carbachol. Inhibition by cyclopropane of catecholamine release was significant compared with the control condition, $P < 0.001$.

mM KCl solution containing 40 mM Tris–HCl (pH 7.4). The reaction was started by adding 0.1 ml of the granule preparation (2.5 mg protein, 450 μg catecholamines) to 3.0 ml of incubation medium containing 150 mM KCl, 4 mM adenosine triphosphate, 2 mM MgSO$_4$,

TABLE 1. Effects of Cyclopropane and Nitrogen on Carbachol-induced Catecholamine Release from Perfused Adrenals*

Agent Added for Second Stimulation†	Carbachol-induced Catecholamine Release (Per Cent of First Stimulation Value, Mean ± SE)	
	Second Stimulation	Third Stimulation
None	83.6 ± 1.8 (n = 12)	65.2 ± 3.1 (n = 6)
Cyclopropane 10 per cent 20 per cent 30 per cent	74.2 ± 3.7‡ (n = 4) 43.5 ± 5.1§ (n = 4) 27.1 ± 2.2§ (n = 4)	68.8 ± 4.8 (n = 3) 62.9 ± 2.3 (n = 4) 64.5 ± 5.7 (n = 4)
Nitrogen 30 per cent	81.0 ± 4.0 (n = 4)	68.3 ± 4.5 (n = 4)

* Adrenals were stimulated twice or three times at 15-min intervals with 0.1 mM carbachol.

† Cyclopropane or nitrogen was added to the perfusate on the second stimulation only.

‡ $P < 0.05$.

§ $P < 0.001$.

and 40 mM Tris-HCl (pH 7.4). Prior to the addition of the granules, the medium was warmed to 37 C in a 30-ml centrifuge tube with a cap and aerated through a cannula with either the 95 per cent O$_2$–5 per cent CO$_2$ mixture or cyclopropane in the aeration mixture for 15 min. Cyclopropane was delivered by a calibrated flowmeter, at a concentration of 10, 20, or 30 per cent. When the reaction was started, aeration of the liquid phase was stopped, but the gas mixture was continued through the gas phase to maintain equilibrium between the gas and liquid phases. After incubation for 10 min, the tubes were cooled in an ice bath and centrifuged at 20,000 × g for 10 min at 2 C, and the catecholamine content in the supernatant was measured.

Results

Spontaneous release of catecholamines during the 4-min period prior to stimulation amounted to 18 ± 1 μg/min (mean ± SE; n = 12). Carbachol-induced catecholamine release during the initial stimulation period was 183 ± 7 μg/min (n = 12); the amounts released during the second and third stimulations were 84 ± 2 per cent (n = 12) and 65 ± 3 per cent (n = 6), respectively, of that released during the initial stimulation. Figure 1 shows the time course of the

experiment and the effects of 25 per cent cyclopropane on spontaneous and carbachol-induced catecholamine release. Cyclopropane had no effect on spontaneous catecholamine release at any concentration tested. Carbachol-induced catecholamine release was inhibited by cyclopropane in a dose-dependent manner (fig. 1, table 1). The inhibition was reversible, since the release on the third stimulation without cyclopropane was restored to the control level. Nitrogen (30 per cent) had no effect on carbachol-induced catecholamine release from the adrenal medulla (table 1). Cyclopropane exerted no action on spontaneous (free of Mg^{++}–ATP) or Mg^{++}–ATP-dependent catecholamine release from isolated adrenal medullary granules (fig. 2).

Discussion

Possible explanations for the increase in plasma catecholamines observed during cyclopropane anesthesia include: 1) increase in sympathetic nerve activity through central mechanisms[14]; 2) exocytosis of granules that contain catecholamines through a direct peripheral action,[9] and 3) increase in catecholamines associated with factors other than sympathoadrenal activity (e.g., reduced biotransformation of catecholamines[15]).

Although the stimulative effect of cyclopropane on the sympathetic system was initially attributed to central mechanisms,[14] recent detailed studies have shown the direct action of cyclopropane on the central sympathetic system to be depressant.[7] Concerning a peripheral catecholamine release mechanism, Ngai et al.[16] reported that cyclopropane had no apparent effect on the spontaneous release of norepinephrine from the dog heart or on the stimulated release of norepinephrine from the cat iris, and Roizen et al.[8] reported that cyclopropane reduced veratridine-induced release of norepinephrine from the isolated guinea-pig vas deferens.

The present experiment, in which we examined the direct effect of cyclopropane on the release of catecholamines from the adrenal medulla, which supplies the major portion of catecholamines in plasma,[1] showed no stimulative action, but rather an inhibition of carbachol-induced release of catecholamines. The inhibition was not due to a decrease in the oxygen concentration in the perfusate, since 30 per cent nitrogen exerted no effect on carbachol-induced catecholamine release. These results and others[8] suggest that the peripheral action of cyclopropane inhibits catecholamine release from both the sympathetic nerve endings and the adrenal medulla, and accordingly throw doubt upon the suggestion that the increase in plasma catecholamine level as a result of anesthesia might be caused by a
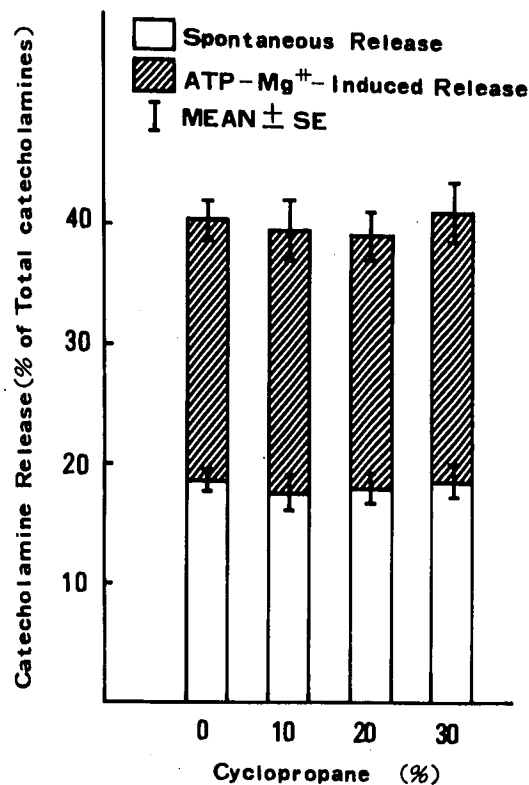

FIG. 2: Effect of cyclopropane on catecholamine release from adrenal medullary granules. Granules were incubated in medium containing various concentrations of cyclopropane in the presence or absence of Mg^{++}–ATP. The ordinate represents the release of catecholamines as a percentage of total catecholamines. For controls, n = 8; for cyclopropane values, n = 5.

direct action on the peripheral catecholamine-release mechanism.[9]

Probable mechanisms involved in the increase in plasma catecholamines during cyclopropane anesthesia may relate to the following: 1) Indirect actions or factors may be involved; Fukunaga and Epstein[7] have suggested that barostatic reflex mechanisms are responsible for sustaining circulation during cyclopropane anesthesia. 2) Although the uptake of catecholamines by the heart has been shown not to be affected by cyclopropane,[17] the pharmacokinetics of the elimination of plasma catecholamines during cyclopropane anesthesia have not been thoroughly investigated; Gardier et al.[15] have shown that catecholamine biotransformation in vitro is reduced by cyclopropane.

Concerning the effect of cyclopropane on catecholamine-containing granules, Seeman[9] has advanced the view that cyclopropane might elicit exocytosis of the catecholamine-containing granules as a result of the membrane-fluidizing effect of the anesthetic. On the other hand, Roizen et al.[8] demonstrated that cyclopropane reduced the release of norepinephrine, but not that of dopamine-β-hydroxylase, from the sympa-

thetic nerve endings. They suggested, as the mechanism involved, that cyclopropane might cause an increase in the affinity of norepinephrine for binding sites on the vesicular membrane, or might have a direct effect on a norepinephrine release mechanism that could be controlled independently of dopamine-β-hydroxylase release. The present results show that cyclopropane has no effect on either spontaneous catecholamine release or Mg^{++}–ATP-induced catecholamine release from isolated adrenal medullary granules. Mg^{++}–ATP-induced catecholamine release has been regarded as a physiologic process.[18] Therefore, it appears impossible that cyclopropane might inhibit catecholamine release resulting from the enhancement of the binding of catecholamines with granules[8] or that the anesthetic might induce exocytosis resulting from the membrane-fluidizing effect on the granules.[9]

From the results obtained in two experimental models, it is concluded that cyclopropane has no effect on spontaneous catecholamine release from the adrenal medulla, has a direct inhibitory effect on stimulated catecholamine release from the adrenal medulla, and has no effect on stimulated adrenal medullary granules. The possible mechanisms involved in the cyclopropane-induced increase in plasma catecholamines and the possible site of the action of cyclopropane in the adrenal medulla remain speculative.

References

1. Roizen MF, Moss J, Henry DP, et al: Effects of halothane on plasma catecholamines. ANESTHESIOLOGY 41:432–439, 1974
2. Skovsted P, Price ML, Price HL: The effects of halothane on arterial pressure, preganglionic sympathetic and barostatic reflexes. ANESTHESIOLOGY 31:507–514, 1970
3. Göthert M, Dreyer C: Inhibitory effect of halothane anesthesia on catecholamine release from the adrenal medulla. Naunyn-Schmiedebergs Arch Pharmacol 277:253–266, 1973
4. Price HL, Linde HW, Jones RE, et al: Sympathoadrenal responses to general anesthesia in man and their relation to hemodynamics. ANESTHESIOLOGY 20:563–575, 1959
5. Perry LB, Van Dyke RA, Theye RA: Sympathoadrenal and hemodynamic effects of isoflurane, halothane and cyclopropane in dogs. ANESTHESIOLOGY 40:465–470, 1974
6. Stokke DB, Christensen NJ, Hole P, et al: Plasma catecholamines during equipotent anaesthesia with cyclopropane and halothane–N_2O in man. Anaesthesist 27:469–474, 1978
7. Fukunaga AF, Epstein RM: Effects of cyclopropane on the sympathetic nervous system and on neural regulation of circulation in the cat. ANESTHESIOLOGY 40:323–335, 1974
8. Roizen MF, Thoa NB, Moss J, et al: Inhibition by cyclopropane of release of norepinephrine, but not dopamine-β-hydroxylase, from the guinea-pig vas deferens. ANESTHESIOLOGY 44:54–56, 1976
9. Seeman P: The membrane actions of anesthetics and tranquilizers. Pharmacol Rev 24:583–655, 1972
10. Oka M., Izumi F, Kashimoto T: Effects of cytoplasmic and microsomal fractions on ATP-Mg^{++} stimulated catecholamine release from isolated adrenomedullary granules. Jpn J Pharmacol 22:207–214, 1972
11. Wada A, Izumi F, Miyashita T, et al: Inhibition by trichlormethiazide of catecholamine release from perfused bovine adrenals. Res Commun Chem Pathol Pharmacol 4:657–698, 1972
12. Weil-Malherbe H: The fluorimetric estimation of catecholamines, Methods in Medical Research. Volume 9. Edited by JH Quastel. Chicago, Yearbook Medical Publishers, 1960, p 130
13. Oka M, Ohuchi T, Yoshida H, et al: The isolation of catecholamine storage granules from adrenal medulla by the membrane filter technique. Life Sci 5:427–432, 1966
14. Price HL, Warden JC, Cooperman LH, et al: Central sympathetic excitation caused by cyclopropane. ANESTHESIOLOGY 30:426–438, 1969
15. Gardier RW, Endahl GL, Hamelberg W: Cyclopropane: Effects on catecholamine biotransformation. ANESTHESIOLOGY 28:677–679, 1967
16. Ngai SH, Diaz PM, Ozer S: The uptake and release of norepinephrine: Effects of cyclopropane and halothane. ANESTHESIOLOGY 31:45–52, 1969
17. Li TH, Laasbery LH, Etsten BE: Effects of anesthetics on myocardial catecholamines. ANESTHESIOLOGY 25:641–649, 1964
18. Pollard HB, Zinder O, Hoffman PG, et al: Regulation of the transmembrane potential of isolated chromaffin granules by ATP, ATP analogs and external pH. J Biol Chem 251:4544–4550, 1976