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MECHANISM WHEREBY HALOTHANE REVERSES THE PRESSOR RESPONSES TO ACETYLCHOLINE

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The initial and secondary components of the biphasic pressor response to acetylcholine in the atropinized dog were analysed separately. Deep halothane anaesthesia reversed the initial pressor response to acetylcholine owing to a decrease in total peripheral vascular resistance in the absence of an increase in cardiac output. The secondary pressor response was not reversed but was suppressed owing to a marked reduction of the increase in cardiac output responsible for this pressor response; total peripheral resistance increased. Conversely, halothane anaesthesia did not block the increase in blood glucose concentration resulting from the injection of acetylcholine. Thus, the change induced by halothane in the secondary pressor phase was apparently a consequence of cardiac depression rather than of adrenal medullary blockade. Compound P-286 (*N*-diethylaminoethyl-*N*-isopentyl-*N'N'*-di-isopropylurea), which produces a change in the pressor response to acetylcholine similar to that induced by halothane, prevents the hyperglycaemia due to acetylcholine. In some experiments, deep halothane anaesthesia depressed the cardiac inotropic but not the chronotropic response to acetylcholine. Such selective blocking action is believed to have a bearing on production of arrhythmias.

Raventós (1956) demonstrated that halothane reversed the pressor response to large doses of acetylcholine in the atropinized dog, and that it depressed the increase in blood pressure caused by splanchnic nerve stimulation. These results suggested that halothane had a sympathetic ganglion-blocking action. *N*-diethylaminoethyl-*N*-isopentyl-*N'N'*-di-isopropylurea (P-286) produces similar effects by specific blockade of the adrenal medulla (Gardier, Abreu, Richards & Herrlich, 1960). Therefore it was of interest to investigate the mechanism of the reversal of the pressor response to acetylcholine during halothane anaesthesia, and to compare it with that of P-286.

METHODS

Adult mongrel dogs of either sex were used. Anaesthesia was induced with methohexitone sodium injected intravenously, in a dose of 10 mg/kg or less. Animals were intubated with a cuffed Magill endotracheal tube. To obtain responses in the absence of halothane, anaesthesia was maintained with a constant infusion of the barbiturate. After lightening of anaesthesia to stage II, the dogs were anaesthetized with halothane and oxygen, using the Forregger F-9 animal apparatus with a semi-closed system. Because this apparatus does not permit estimation

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of the concentrations of halothane in the inspired air, the levels of halothane anaesthesia were categorized as " light," " moderate " and " deep " in accordance with the levels of blood pressure and heart rate. Atropine sulphate was given in doses of 1 mg/kg, intravenously. Compound P-286 (*N*-diethylaminoethyl-*N*-isopentyl-*N'N'*-di-isopropylurea) was usually given in a total dose of 6 mg/kg, intravenously, in divided doses of 2 mg/kg over a period of 15 min. Acetylcholine chloride was given intravenously in a dose of 1 mg/kg. Adrenaline hydrochloride was injected intravenously in a dose of 5 μg/kg.

Femoral arterial blood pressure was measured with a Statham P23AC transducer and recorded on a Grass Model 5 ink writing polygraph.

Cardiac output was determined by the dye (Evans blue, T-1824) dilution technique (Hamilton, Moore, Kinsman & Spurling, 1932) and total peripheral vascular resistance was calculated as the mean femoral blood pressure (diastolic plus one third of the pulse pressure) divided by the cardiac output in ml./min/100 g of body weight. Lead II of the electrocardiogram was recorded simultaneously with blood pressure, and stroke volume was derived from the cardiac output and pulse rate.

The concentration of blood glucose was recorded continuously with ante-brachial venous blood, using a Technicon Auto-analyzer.

The data were evaluated by analysis of variance (Snedecor, 1956); *P* values of 0.05 or less were considered significant.

RESULTS

The " nicotinic " action of acetylcholine in the atropinized dog anaesthetized with barbiturates was characterized by an early transient pressor response followed by a slight fall and then a prolonged rise in pressure. Presumably, the initial rise results from ganglionic stimulation and the secondary increase from stimulation of the adrenal medulla. These blood pressure responses to large doses of acetylcholine also occurred in the atropinized animal very lightly anaesthetized with halothane. If the halothane anaesthesia was deepened the initial transient rise in blood pressure

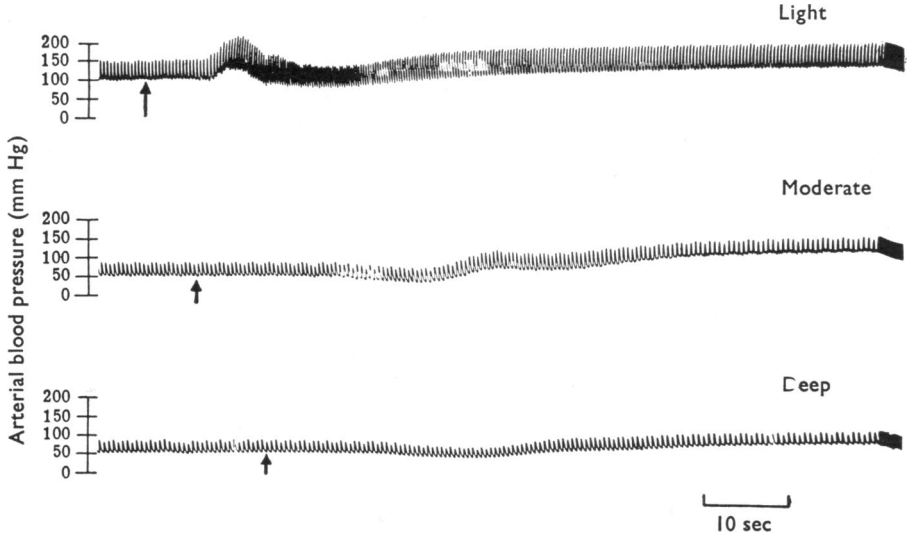

Fig. 1. Arterial blood pressure responses to acetylcholine in atropinized dogs during anaesthesia at different levels with halothane. Depth of anaesthesia shown on right. Acetylcholine chloride (1 mg/kg, intravenously) was injected at the arrows. Records with Statham transducers.

was abolished or reversed while the secondary pressor effect was essentially unaffected. Further deepening of anaesthesia with halothane almost completely eliminated the secondary pressor response but the reversal of the initial pressor effect remained or was exaggerated. This pattern of response to acetylcholine in dogs anaesthetized with halothane is illustrated in Fig. 1.

The actions of acetylcholine in dogs anaesthetized with the ultra-short acting barbiturate, methohexitone, and lightly and deeply with halothane were analysed (Tables 1, 2 and 3).

Arterial blood pressure. The blood pressure was lowered during halothane anaesthesia (Table 1, uppermost section).

TABLE 1

CARDIOVASCULAR RESPONSES TO ACETYLCHOLINE IN ATROPINIZED DOGS

Acetylcholine was given in a dose of 1 mg/kg, intravenously. Values are means with standard errors from five experiments. Blood pressures are in mm Hg, heart rates in beats/min. * Value significantly different ($P<0.05$) from control

	Anaesthetic agent		
	Methohexitone sodium	Halothane (light)	Halothane (deep)
Before acetylcholine			
Systolic blood pressure	169± 7·4	138± 6·7	102±6·2
Diastolic blood pressure	108± 4·5	93± 6·3	58±4·2
Heart rate	166± 4·5	133± 7·5	120±3·5
During initial pressor response to acetylcholine			
Systolic blood pressure	*213±13·5	156±20·3	*78±6·3
Diastolic blood pressure	117± 9·6	84±15·1	*37±3·1
Heart rate	*199±10·2	160±19·7	124±4·8
During secondary pressor response to acetylcholine			
Systolic blood pressure	*235± 9·3	*231± 9·8	*138±6·1
Diastolic blood pressure	*144± 6·4	*151± 5·3	*94±6·4
Heart rate	172±10·2	144±13·6	122±3·9

TABLE 2

CARDIOVASCULAR RESPONSES TO ACETYLCHOLINE IN ATROPINIZED DOGS ANAESTHETIZED WITH HALOTHANE

Values are means with standard errors from five experiments. * Values significantly different ($P<0.05$) from control. † For units see Methods

	Control	During secondary pressor response to acetylcholine (1 mg/kg intravenously)
Light anaesthesia		
Systolic blood pressure (mm Hg)	137 ± 6·4	*203 ±14·4
Diastolic blood pressure (mm Hg)	96 ± 5·8	*139 ± 9·5
Heart rate (beats/min)	148 ± 8·6	162 ±16·6
Cardiac output (l./min)	2·57± 0·13	3·40± 0·45
Stroke volume (ml.)	17·6 ± 1·4	21·5 ± 2·9
Peripheral resistance†	7·96± 0·39	8·63± 1·04
Deep anaesthesia		
Systolic blood pressure (mm Hg)	91 ± 9·1	117 ±21·9
Diastolic blood pressure (mm Hg)	55 ± 5·9	80 ±16·9
Heart rate (beats/min)	130 ±11·4	136 ±12·1
Cardiac output (l./min)	1·85± 0·29	2·09± 0·29
Stroke volume (ml.)	15·0 ± 3·2	15·8 ± 2·7
Peripheral resistance†	7·08± 0·77	8·75± 1·73

TABLE 3

CARDIOVASCULAR RESPONSES TO ACETYLCHOLINE IN ATROPINIZED DOGS ANAESTHETIZED DEEPLY WITH HALOTHANE

Values are means with standard errors from three experiments. † For units see Methods

	Control	During initial depressor response to acetylcholine (1 mg/kg)
Systolic blood pressure (mm Hg)	98 ±10·1	83 ±14·5
Diastolic blood pressure (mm Hg)	67 ± 9·3	57 ±17·4
Heart rate (beats/min)	117 ±13·3	157 ±12·0
Cardiac output (l./min)	1·22± 0·29	1·32± 0·20
Stroke volume (ml.)	10·5 ± 2·2	8·5 ± 1·5
Peripheral resistance†	7·34± 0·91	5·33± 0·76

The initial pressor response to acetylcholine during barbiturate anaesthesia was brought about predominantly by an increase in systolic pressure. This initial pressor effect was extremely susceptible to suppression by halothane; the systolic pressure increment was reduced and the mean diastolic pressure actually fell during light anaesthesia, whereas deep halothane anaesthesia led to initial decrements in both systolic and diastolic pressure following injection of acetylcholine.

The secondary pressor effect was more resistant to halothane. Even during deep halothane anaesthesia in the experiments listed in Table 1, acetylcholine elicited statistically significant increases in systolic and diastolic pressures.

Cardiac output and total peripheral vascular resistance. The secondary pressor response to acetylcholine, which was statistically significant during light halothane anaesthesia, was markedly reduced by deepening the anaesthetic level with halothane (Table 2). This pressor response to acetylcholine was largely a result of an increase in cardiac output, and likewise the reduction in this pressor response by deepening the anaesthesia with halothane was essentially due to a smaller increase in cardiac output (Table 2).

During the secondary pressor response to acetylcholine, there was a small increase in peripheral resistance during light halothane, and a slightly greater increase during deep halothane anaesthesia (Table 2). Thus, the increase in peripheral resistance contributes relatively more to the secondary pressor response during deep halothane anaesthesia than it does during light anaesthesia. This result—increased peripheral resistance—differs from that of previous experiments with pentobarbitone in which the peripheral resistance decreased during both the initial and secondary pressor responses induced by acetylcholine (Gardier, James, Johnson, Richards & Roesch, 1963). Nevertheless, it is apparent that the magnitude of the secondary pressor response to acetylcholine during halothane anaesthesia was chiefly determined by the increase in cardiac output. Apart from altering the responses to acetylcholine, deepening the level of anaesthesia with halothane decreases the "control" cardiac output by 25 to 50% and slightly lowers total peripheral vascular resistance (Table 2).

The reversal of the initial phase of the pressor response to acetylcholine during deep halothane anaesthesia was caused by a decrease in peripheral resistance (Table 3).

Stroke volume. An increase in stroke volume contributed substantially to the secondary pressor response to acetylcholine in dogs lightly anaesthetized with

halothane, but with deep halothane anaesthesia there was only a trivial increase in stroke volume (Table 2).

With deep halothane anaesthesia, acetylcholine caused an initial depressor response and, although the cardiac output increased, the stroke volume fell (Table 3). Thus, the elevated heart rate was responsible for the increased cardiac output (Table 3). Here it may be inferred that halothane prevented the cardiac inotropic but not the chronotropic response to catechol amines released by acetylcholine.

TABLE 4

THE EFFECT OF ACETYLCHOLINE IN RAISING THE CONCENTRATION OF BLOOD GLUCOSE IN ATROPINIZED DOGS

Values are means with standard errors. The results in (*A*) are from the same dogs which provided the results in Table 1; those in (*B*) are from a separate group of five dogs

	Anaesthesia	Increase in blood glucose concentration (mg/100 ml.) after		
(*A*)		Acetylcholine (1 mg/kg)		
	Methohexitone	20·2±3·1		
	Halothane (light)	17·5±2·9		
	Halothane (deep)	19·1±3·2		
(*B*)		Acetylcholine (1 mg/kg)		Adrenaline (5 μg/kg)
		Before P-286	After P-286	After P-286
	Methohexitone	33·2±6·0	4·6±1·4	18·2±0·7

Blood glucose. The hyperglycaemic response to large doses of acetylcholine in the atropinized dog was not prevented by halothane but was by P-286. The results are shown in Table 4, and they suggest that halothane did not prevent the release of catechol amines from the adrenal medulla.

DISCUSSION

The superficial pharmacological similarities between halothane and P-286 mentioned in the Introduction disappear when the results are analysed in detail. These results demonstrate that halothane does not inhibit the discharge of adrenaline from the adrenal medulla induced by acetylcholine, since the blood glucose level is increased, but the heart does not respond to the adrenaline that is liberated.

In some experiments with deep halothane anaesthesia (Table 3), although a negative cardiac inotropic response was invoked by acetylcholine during the initial phase of its action, a positive chronotropic response persisted. It is possible that this selective blocking action is pertinent to the production of arrhythmias during halothane anaesthesia.

Production of arrhythmias during the light planes of anaesthesia was often found; many experiments were lost due to ventricular fibrillation when acetylcholine was injected into dogs thus anaesthetized, but this never happened during deep anaesthesia. It is difficult to decide whether the fibrillation resulted from stimulation of sympathetic ganglia by acetylcholine or was due to circulating adrenaline released from the adrenal medulla. The initial pressor response to acetylcholine was present

within 10 sec of intravenous injection, and the secondary pressor response started 25 to 40 sec following injection. Since fibrillation ensued within 20 sec, it was probably caused by local liberation of catechol amines in the heart. Price, Lurie, Jones, Price & Linde (1958) found that the local liberation of catechol amines was more important than the level of circulating catechol amines for the production of arrhythmias during anaesthesia with cyclopropane.

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REFERENCES

GARDIER, R. W., ABREU, B. E., RICHARDS, A. B. & HERRLICH, H. C. (1960). Specific blockade of the adrenal medulla. *J. Pharmacol. exp. Ther.*, **130**, 340–345.

GARDIER, R. W., JAMES, E. A. Jr., JOHNSON, P. C., RICHARDS, A. & ROESCH, R. P. (1963). Vasodepression induced by acetylcholine in the atropinized dog. *Brit. J. Pharmacol.*, **20**, 579-585.

HAMILTON, W. F., MOORE, J. W., KINSMAN, J. M. & SPURLING, R. G. (1932). Studies on the circulation. IV Further analysis of the injection method and of changes in hemodynamics under physiological and pathological conditions. *Amer. J. Physiol.*, **99**, 534–551.

PRICE, H. L., LURIE, A. A. JONES, R. E., PRICE, M. L. & LINDE, H. W. (1958). Cyclopropane anesthesia. II Epinephrine and norepinephrine in initiation of ventricular arrhythmias by carbon dioxide inhalation. *Anaesthesiology*, **19**, 619–630.

RAVENTÓS, J. (1956). The action of fluothane—a new volatile anaesthetic. *Brit. J. Pharmacol.*, **11**, 394–409.

SNEDECOR, C. W. (1956). *Statistical Methods*, 6th ed., p. 237. Iowa State College Press: Ames.