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ACTION OF COCAINE AND CHRONIC SYMPATHETIC DENERVATION ON VAGAL ESCAPE

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SUMMARY

1. The effect of cocaine has been studied on vagal escape and on the tachycardia due to vagal stimulation in the atropinized dog. All the dogs were submitted to acute cervical section of the spinal cord and acute or chronic sympathetic denervation.

2. Cocaine, 5 mg/kg or 40 μg/kg/min, I.V., induces a significant enhancement of the ventricular escape. The effects of a continuous infusion of cocaine are more reproducible than those of a single injection of the drug.

3. Cocaine, 40 μg/kg/min, I.V., potentiates the tachycardia due to vagal stimulation in the atropinized dog.

4. Chronic thoracic sympathectomy markedly retards the recovery of the ventricular rate from the inhibitory action of the vagus. Under this condition, the infusion of cocaine does not significantly enhance the ventricular escape.

5. These findings suggest that an adrenergic mechanism located at the sympathetic nerves supplying the heart is substantially involved in the phenomenon of vagal escape.

INTRODUCTION

Hoffman, Hoffman, Middleton & Talesnik (1945) suggested that an adrenergic mechanism located in the heart itself might be involved in the phenomenon of vagal escape. Experimental evidence in favour of this assumption was provided by Friedman & Campos (1960), who showed that

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ventricular escape is significantly retarded by previous reserpinization or treatment with choline xylyl ether bromide, an adrenergic neurone blocker. Roberts & Stadter (1960) also showed that the retarded ventricular escape in the catecholamine depleted heart could be enhanced by replenishment of noradrenaline.

Since the above-mentioned data suggest that, in the course of vagal escape, catecholamines are liberated from stores in the heart, the present study was carried out to see whether cocaine, known to potentiate peripheral responses to catecholamines (Fröhlich & Loewi, 1910), enhanced the recovery of the ventricular rate from the inhibitory action of the vagus. To gain some insight about the sites of release of cardiac catecholamines during vagal escape, this phenomenon was also studied in dogs under chronic sympathetic denervation. A preliminary report has been published elsewhere (Urquilla & Campos, 1966).

METHODS

Experimental procedure. Mongrel dogs of either sex, weighing between 7 and 19 kg, were anaesthetized with pentobarbitone sodium, 30 mg/kg, I.P., and maintained under artificial respiration. The blood pressure was recorded from the left carotid artery by means of a Statham pressure transducer and a Grass polygraph. A femoral vein was cannulated for the injection of drugs. Spinal transection (C3–C4), bilateral stellate ganglionectomy and removal of the thoracic sympathetic chain down to T 4 were performed in all the animals. An equilibration period of at least 1 hr was allowed before the experiment was started. In those animals with chronic sympathetic denervation, the ganglionectomy and removal of the sympathetic chain were performed under aseptic conditions. These dogs were used 2 weeks after they were submitted to the operation. Just before the experiment acute spinal transection was performed.

In all the animals both vagi were dissected free from their carotid sheaths and sectioned. The peripheral end of the right vagus was fixed to platinum electrodes for stimulation. Supramaximal square wave pulses of 0·2 msec duration and a frequency of 60 c/s were applied to the nerve. The stimulation was maintained over a period of 2 min and a 10 min interval was allowed for rest. The nerve was prevented from drying by maintaining it in a well formed by the surrounding muscles sutured on to an iron ring which was fastened on a stand. The well was covered with cotton wool moistened with saline. Care was taken to keep the rectal temperature of the animal at approximately 37° C by means of a heating pad.

The ventricular rate was measured for each of the twelve successive 10 sec intervals of the 2 min period of stimulation. The values were expressed as a percentage of the 10 sec prestimulation value and accumulated. Comparisons of the various treatments were made on basis of the final cumulative percentage value obtained over the 2 min period of stimulation. An acceleration of the escape pattern was indicated by an increase in this value. Conversely a retardation of the escape pattern was indicated by a decrease in this value in comparison with the control. In order to facilitate comparison of the effects of treatments, means of the final cumulative percentage values were expressed as percentages of untreated control group values (Campos & Friedman, 1963).

Cocaine HCl (E. Merck) was administered in a dose of 5 mg/kg I.V. in one group. In the other groups the drug was infused I.V. at a rate of 40 μg/kg/min in a volume of saline of 0·38 ml./min. Atropine sulphate (E. Merck), 0·25 mg/kg I.V., was given in one injection

plus 5 μg/kg/min by constant infusion. All the drugs were dissolved in saline. Controls received saline alone. Schedules for administration of drugs are given in the Results.

Statistical methods. Statistical methods and terminology are those used by Snedecor (1956).

RESULTS

Effect of cocaine on ventricular escape. Cocaine was administered at two dose levels since the relatively large dose of the drug (5 mg/kg) might be exerting actions related to its local anaesthetic activity. A group of five dogs was submitted to stimulation of the right vagus to obtain control curves for vagal escape. Then a single injection of cocaine, 5 mg/kg

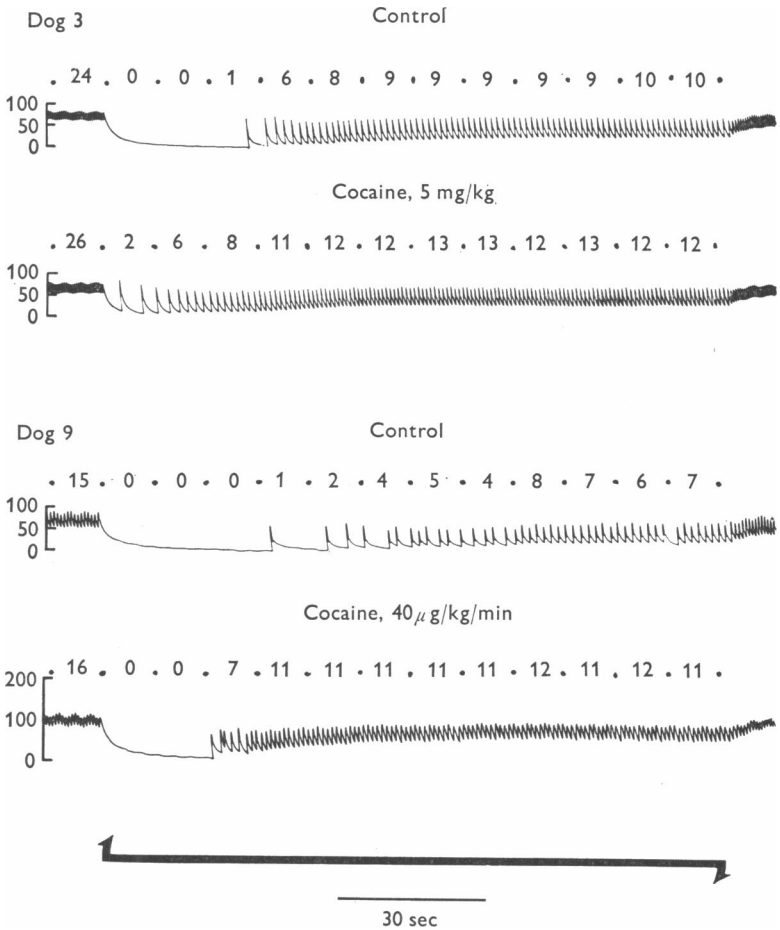

Fig. 1. Action of cocaine on vagal escape. The horizontal bar indicates the 2 min period of vagal stimulation. Figures above tracings correspond to heart rate for each 10 sec interval. Observe that cocaine enhances the recovery of the ventricular rate from the inhibitory action of the vagus either by a single injection (second tracing from top) or by a constant infusion (bottom).

I.V., was administered 5 min before the second stimulation of the vagus. The two upper records in Fig. 1 illustrate a typical experiment. The first record is the control and the one below shows the effect of cocaine on the course of vagal escape. This treatment shortened the periods both of systolic arrest and of hypotension during vagal stimulation and enhanced the recovery of the cardiac rate (see Table 1). Another group of five dogs received an intravenous infusion of cocaine, 40 μg/kg/min, during the stimulation of the vagus. The two lower records in Fig. 1 illustrate one of these experiments. The first record is the control and the next one down

TABLE 1. Effect of cocaine and chronic sympathetic denervation on vagal escape over a 120 sec period of stimulation

Treatment	No. of dogs	Cumulative percentage of pre-stimulation value (heart rate, beats/10 sec)	Cumulative heart rate as % of control
		Mean ± S.E. of mean	
Control	10	41·7 ± 2·7	100
Cocaine 5 mg/kg	5	61·5 ± 5·6*	146
Cocaine 40 μg/kg/min	5	56·4 ± 3·5*	135
Denervation	3	16·2 ± 5·6*	39
Denervation + cocaine 40 μg/kg/min	3	18·5 ± 5·4*	44

* Different from controls, $P < 0{\cdot}01$.

shows the effect of the infusion of cocaine started 5 min before the vagal stimulation and maintained throughout the experiment. As before, the periods of systolic arrest and of hypotension are reduced and the recovery of cardiac rate is accelerated. The influence of cocaine on the time course of vagal escape is shown in Fig. 5. A significant shift of the curve to the left is observed when vagal escape occurs either after a single injection of cocaine or during a constant infusion of the same drug. In the latter case, unlike the former, subsequent vagal stimulations at 15 and 25 min after the beginning of the infusion of cocaine did not reveal significant changes in the effect of the drug.

Effect of cocaine on the tachycardia due to vagal stimulation in the atropinized dog. The atropinization was initiated at least 10 min before vagal stimulation and a constant infusion of atropine was maintained throughout the experiment. In agreement with previous findings (Jourdan & Nowak, 1934), vagal stimulation then elicited tachycardia instead of the usual inhibitory effect. The infusion of cocaine, together with atropine, potentiated the tachycardia due to vagal stimulation and a rise of the arterial pressure appeared (Fig. 2). The increase of cardiac rate in the presence of cocaine averages 132% over the control value ($P < 0{\cdot}02$;

Table 2). The time course of the cardiac rate under these conditions is shown in Fig. 3. A pronounced shift of the curve to the left after treatment with cocaine is observed, which is similar at 10, 20 and 30 min after the beginning of the infusion of the drug.

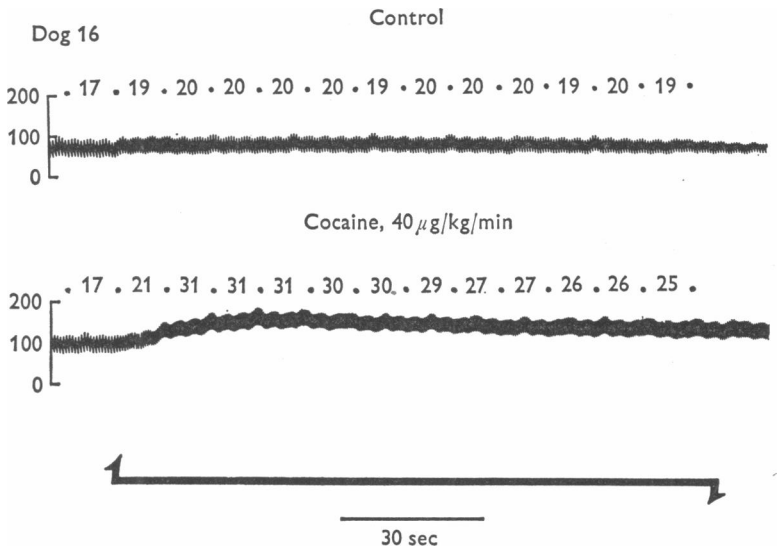

Fig. 2. Action of cocaine on the tachycardia due to vagal stimulation in the atropinized dog. The horizontal bar indicates the 2 min period of stimulation. Figures above tracings correspond to heart rate for each 10 sec interval. Note that cocaine enhances this type of response in the heart.

TABLE 2. Effect of cocaine on the tachycardia induced by a 120 sec period of vagal stimulation in the atropinized dog

Treatment	No. of dogs	Cumulative percentage of pre-stimulation value (heart rate, beats 10/sec above normal)	Cumulative heart rate increase (%)
		Mean ± S.E. of mean	
Vagal stimulation	6	19·0 ± 3·7	100
Vagal stimulation + cocaine, 40 μg/kg/min	6	44·5 ± 5·3*	232

* Different from control vagal stimulation, $P < 0{\cdot}02$.

Influence of chronic sympathetic denervation on ventricular escape. In three chronically sympathectomized dogs, as illustrated in Fig. 4, the stimulation of the vagus induced a cardiac arrest of prolonged duration and a retardation of vagal escape (see also Table 1). The infusion of cocaine, 40 μg/kg/min, induced no significant enhancement of the recovery of

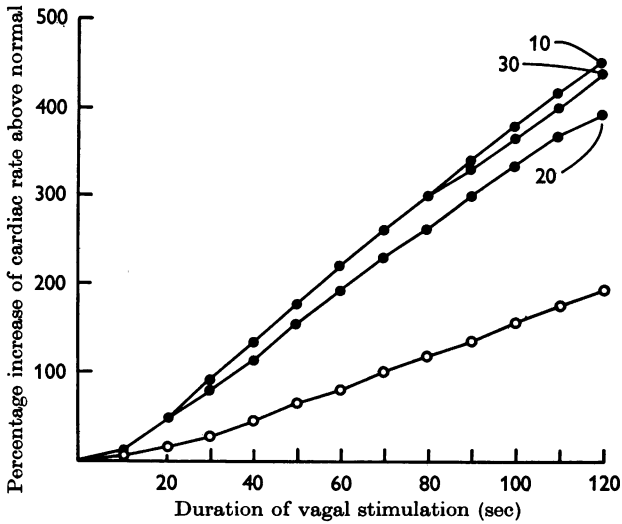

Fig. 3. Action of cocaine on the time course of the tachycardia due to vagal stimulation in the atropinized dog. The heart rate increase is plotted against the time of vagal stimulation. ○—○—○ controls (six animals); ●—●—● cocaine 40 μg/kg/min (six animals). Figures at the end of the curves indicate the time of vagal stimulation at 10, 20 and 30 min after the beginning of the infusion of cocaine.

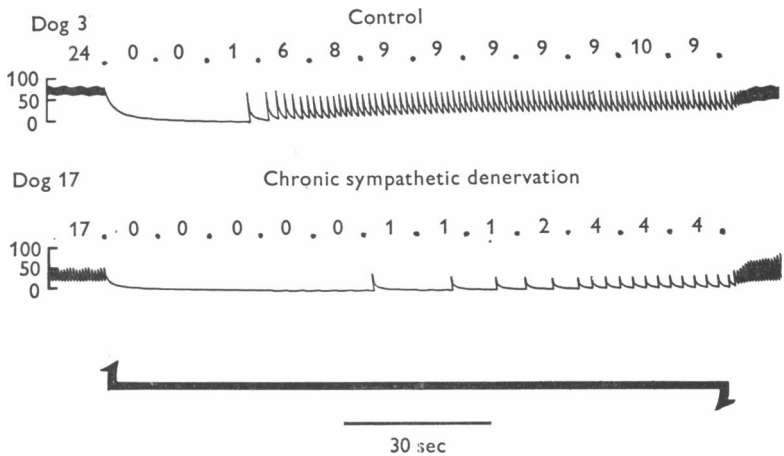

Fig. 4. Effect of chronic sympathetic denervation on vagal escape. The upper tracing shows ventricular escape in a dog submitted to acute thoracic sympathectomy (control) and the lower one shows ventricular escape in a dog submitted to chronic thoracic sympathectomy beforehand. Note that in the latter case the ventricular escape is markedly retarded. The horizontal bar indicates the 2 min period of stimulation. Figures above tracings correspond to heart rate for each 10 sec interval.

cardiac rate ($P > 0{\cdot}05$; Table 1). The time course of vagal escape in chronically sympathectomized dogs, before and after treatment with cocaine, is shown in Fig. 5. In both instances similar flattened curves are obtained because of the marked retardation of vagal escape and no significant influence of cocaine is observed.

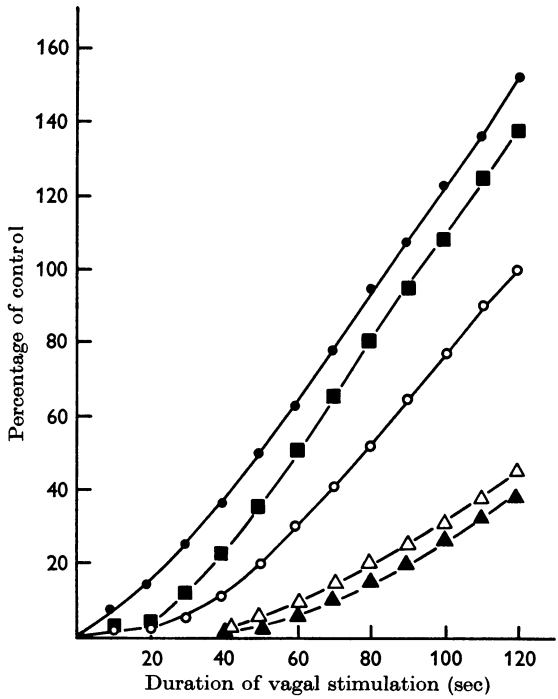

Fig. 5. Action of cocaine and chronic sympathetic denervation on the time course of vagal escape. The cumulative percentage, expressed as the percentage of the final (2 min) cumulative percentage of the untreated control, is plotted against the time of vagal stimulation. ●—●—● cocaine 5 mg/kg I.V. (five animals); ■—■—■ cocaine 40 μg/kg/min (five animals); ○—○—○ controls (ten animals); △—△—△ chronic sympathetic denervation plus cocaine 40 μg/kg/min (three animals); ▲—▲—▲ chronic sympathetic denervation (three animals). Note that cocaine enhances the recovery of the cardiac rate in the normal dog, but not in the chronically sympathectomized dog. Chronic sympathectomy markedly retards the time course of vagal escape.

DISCUSSION

Several workers (Heymans & Benatti, 1949; Ginzel & Kottegoda, 1953; Burn, Leach, Rand & Thompson, 1959) have shown that acetylcholine induces sympathomimetic responses in the atropinized heart, presumably by the activation of sympathetic structures (ganglia and/or chromaffin tissue) located in the myocardium. It has also been pointed out that

acetylcholine liberates an adrenaline-like substance from cardiac tissues (Hoffman *et al.* 1945), which has been identified as noradrenaline (Richardson & Woods, 1959; Löffenholz, 1967). Since the stimulation of the peripheral end of the vagus in the atropinized dog induces cardioacceleration, the acetylcholine liberated at the nerve endings could be in turn liberating adrenergic amines from the heart (Hoffman *et al.* 1945; Middleton, Middleton & Toha, 1949). A similar local action of acetylcholine has also been pointed out more recently by Leaders (1963). All these findings suggest that a mechanism of this sort might be involved, in the absence of atropine, in the phenomenon of vagal escape. Evidence in favour of this assumption has been presented previously (Friedman & Campos, 1960; Roberts & Stadter, 1960; Campos & Friedman, 1963). In the present work it is shown that cocaine significantly enhances the recovery of the heart from the inhibitory action of the vagus, which is in agreement with recent findings by Moore (1967) in the open-chest dog. As cocaine is known to potentiate adrenergic responses (Fröhlich & Loewi, 1910), the above-mentioned findings give further support to the assumption that an adrenergic mechanism located in the heart itself is involved in the escape phenomenon.

It has been shown that the stimulant action of acetylcholine in the atropinized heart is abolished by previous reserpinization (Alvarado, Middleton & Beca, 1961) or substantially reduced by chronic sympathetic denervation (Cabrera, Cohen, Middleton, Utano & Viveros, 1966), which suggests that acetylcholine induces cardioacceleration through the release of catecholamines from sympathetic nerves supplying the heart. In the case of vagal escape catecholamines from the same site might be released by the endogenous acetylcholine. The possibility has been tested in the present work by studying the effect of chronic sympathetic denervation which induces depletion of cardiac catecholamines (Goodall & Kirshner, 1956; Cooper, Gilbert, Bloodwell & Crout, 1961). The procedure markedly impaired the recovery of the heart from the inhibitory action of the vagus, suggesting that the catecholamines liberated during vagal escape originate for the most part from the sympathetic nerves supplying the heart. This is in agreement with the findings of Cabrera *et al.* (1966) showing that the cardiostimulant action of acetylcholine in the cat heart requires the presence of intact post-ganglionic sympathetic fibres. The relatively poor recovery of the heart from the inhibitory action of the vagus which does occur in the chronically sympathectomized dog might be explained on the basis that some sympathetic fibres remain intact after the thoracic ganglionectomy (Cooper *et al.* 1961) and/or that some uptake of circulating catecholamines occurs in the chronically denervated dog heart (Potter, Cooper, Willman & Wolfe, 1965). However, an extraneuronal binding of noradrenaline

(Fischer, Kopin & Axelrod, 1965) does not seem to be important for the stimulant action of acetylcholine to occur in the heart since an infusion of noradrenaline fails to restore this action in the chronically sympathectomized cat heart (Cabrera *et al.* 1966). In view of these findings, the small portion of catecholamines contained in the remaining intact sympathetic fibres after chronic sympathectomy would be available for release during the course of vagal escape.

Blockade of uptake of catecholamines by cocaine has been shown by several workers (Whitby, Hertting & Axelrod, 1960; Muscholl, 1961; Dengler, Spiegel & Titus, 1961). Cocaine could be enhancing adrenergic responses during vagal escape by blocking the uptake into the axonal terminal of the catecholamines liberated during the occurrence of the phenomenon. This type of blockade would provide more of the liberated amines to the adrenergic receptor sites (Furchgott, Kirpekar, Rieker & Schwab, 1963). Moreover, our findings show that cocaine does not enhance the recovery of the heart from the inhibitory action of the vagus in the chronically sympathectomized dog, suggesting that the presence of catecholamines is necessary for the action of cocaine to occur during vagal escape.

The possibility that the observed effects of cocaine are due to blockade of nervous or synaptic transmission seems very unlikely because cocaine under similar conditions significantly enhances the tachycardia due to vagal stimulation in the atropinized dog and since, as it has been shown in the present work, it does not modify the inhibitory action of the vagus in the chronically sympathectomized dog.

All these findings strongly suggest that an adrenergic mechanism located at the sympathetic nerves supplying the heart is involved in the escape phenomenon.

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