

## THE EFFECT OF INTRA(CEREBRO)VENTRICULAR RESERPINE ON THE ACETYLCHOLINE CONTENT OF THE HEART, ILEUM AND HYPOTHALAMUS OF THE DOG

BY

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The effect of injection of reserpine into the cerebral ventricles on the acetylcholine contents of the sino-atrial node, ileum and hypothalamus of the dog was studied in ten dogs. Another group of five dogs served as a control. The effect of intravenous administration of reserpine, in the same dose as given intracerebroventricularly, was also studied on the acetylcholine content of these tissues in five dogs. General sedation, bradycardia, miosis, salivation, emesis and purgation were looked for. Tissues were removed 1 hr after administration of reserpine for estimation of acetylcholine content, which was increased in all the tissues studied. The increase in the peripheral tissues was greater than in the hypothalamus. The increase in the acetylcholine content was not quantitatively related to the other effects of reserpine. The increase in the acetylcholine content of the sino-atrial node and the ileum and also the peripheral effects observed on intracerebroventricular administration of reserpine can be attributed to its central action. With the same dose of reserpine given intravenously the acetylcholine content of the sino-atrial node was significantly increased, while that of the hypothalamus and ileum was not.

Anand, Dua & Malhotra (1957) showed that reserpine not only depressed the sympathetic centres in the diencephalon but also facilitated the parasympathetic centres. Malhotra & Pundlik (1959) observed that intravenous reserpine increased the acetylcholine content of the frontal and the temporal lobes, the cerebellum, the spinal cord and the hypothalamus of the dog. They suggested that this effect might be in some way related to the facilitation of the parasympathetic centres in the brain. Giarmar & Pepeu (1962) showed that, in rats, intraperitoneal reserpine caused a small but statistically significant increase in acetylcholine content of the cerebrum. Recently Malhotra & Das (1962) observed that intravenous reserpine increased the acetylcholine content not only of the hypothalamus but also of the ileum, the sino-atrial node, the right auricle and the right ventricle in the dog. The increase in acetylcholine content of the peripheral tissues was greater than in the hypothalamus. It was suggested that the parasympathetic activity observed with reserpine—bradycardia and purgation—might be related to the increase in the acetylcholine content of the peripheral organs. The present study was designed to find out whether the predominant peripheral parasympathetic effects of reserpine on the heart, intestinal

motility and pupillary size and also the increase in the acetylcholine content of the sino-atrial node and the ileum were central or peripheral in origin. The drug was administered into the lateral cerebral ventricle through a chronically implanted Collison's cannula, so that it was acting only on the central nervous system.

#### METHODS

Twenty mongrel dogs of either sex, weighing 6 to 13.5 kg, were used ; five served as controls, ten received reserpine into a cerebral ventricle, while in five the same dose of reserpine was given intravenously. With aseptic precautions a Collison's cannula was implanted into a lateral cerebral ventricle, using ether anaesthesia, by the method of Feldberg & Sherwood (1953). A hole was drilled through the skull at a point 0.2 to 0.3 in. on either side of the midline one-third of the way back from a line joining the occipital protuberance to the anterior orbital margin. After 24 hr from implantation of the cannula, reserpine (0.75 mg in 0.4 ml. of 0.9% saline) was injected into the lateral ventricle through the cannula. In dogs of the control group, 0.4 ml. of 0.9% saline was injected into the lateral ventricle, while in those of the other group, 0.75 mg of reserpine was given intravenously.

The degree of sedation, the heart rate, defaecation, emesis, miosis and salivation, if any, were noted in all dogs. The animals were bled to death during ether anaesthesia 1 hr after administration of reserpine or of 0.9% saline and the skull, the abdomen and the chest were opened simultaneously. The following tissues were removed and immediately transferred to weighing bottles kept in freezing mixtures: (a) an annular piece of the proximal part of the ileum containing all its layers ; (b) the hypothalamus ; and (c) an area of the sino-atrial node (the upper two-thirds of the sulcus terminalis). Acetylcholine was extracted from the tissues and assayed by the method of Nachmansohn, as described by Anand (1952). The control experiments were interspersed between the experiments with reserpine. Four dogs, in which the cannula was not in the lateral ventricle as seen from the track in the cerebrum, were not included in the results.

#### RESULTS

The acetylcholine contents of the hypothalamus, the ileum and the sino-atrial node of the heart of all animals are given in Table 1. The greatest concentration of acetylcholine was present in the sino-atrial node, being more than twice that of the ileum and the hypothalamus. Reserpine increased the acetylcholine contents of all the tissues studied, the increases being statistically significant. The increases in the acetylcholine contents of the ileum and the sino-atrial node were greater (42.8% and 40.9% respectively) than that of the hypothalamus (22.3%). Some of the general effects of intracerebroventricular administration of reserpine are given in Table 2. Miosis, sedation and bradycardia were constant features. The degree of the general effects was, however, not related quantitatively to the acetylcholine content of the tissue concerned. Saline (0.4 ml.) produced no detectable effect except that at times there were a few licking movements and transient tachycardia and tachypnoea. Tables 3 and 4 show the effects of intravenous injection of reserpine on the acetylcholine contents of different tissues and on the general responses. Though with 0.75 mg of reserpine given intravenously there was some increase in the acetylcholine content of the tissues, it was statistically insignificant for the hypothalamus and the ileum. For the sino-atrial node, however, the increase was 17.9%, compared to 40.9% with intracerebroventricular administration. The general responses observed were inconstant and very slight.

TABLE 1

## ACETYLCHOLINE CONTENTS OF DIFFERENT TISSUES OF THE DOG

Results are expressed in  $\mu\text{g/g}$  of tissue. Probability of no difference between means of treated and untreated animals was calculated by the "t" test

No. of expt.	Weight of dog (kg)	Tissue		
		Hypothalamus	Ileum	Sino-atrial node
<i>Control</i>				
1	9.5	4.207	3.330	8.130
5	6.5	3.202	3.225	6.152
8	5.8	3.062	3.750	7.625
13	4.5	2.015	2.741	7.834
18	9.0	2.500	3.750	7.526
	Mean	2.997	3.359	7.453
	Standard deviation	±0.824	±0.425	±0.847
<i>Treated with reserpine</i>				
3	6.0	4.456	6.195	16.980
4	8.0	5.000	8.095	16.290
10	10.5	4.120	5.000	9.078
11	12.0	3.213	4.709	9.376
12	10.5	3.133	3.326	8.660
14	13.0	3.966	3.026	11.170
15	7.0	3.500	4.320	8.790
16	9.5	2.831	3.104	6.703
17	8.0	3.400	3.980	8.590
19	9.0	3.250	3.306	9.326
	Mean	3.683	4.806	10.496
	Standard deviation	±0.680	±1.189	±3.368
	<i>P</i>	<0.05	<0.05	<0.01
	Mean increase in acetylcholine content	22.3%	42.8%	40.9%

TABLE 2

## SOME GENERAL EFFECTS OF RESERPINE IN THE DOG

Each of the dogs showed transient initial tachycardia. + = Slight; ++ = moderate; +++ = considerable

No. of expt.	Weight of dog (kg)	Heart rate (beats/min)		Defaecation	Salivation	Emesis	Miosis	Behavioural changes
		Control	Minimum after reserpine					
3	6.0	108	82	No	Yes	Yes	+++	Excitement followed by light sedation
4	8.0	74	56	Yes	No	No	++	Sedation
10	10.5	140	106	Yes	Yes	No	++	Sedation
11	12	95	52	Yes	Yes	Yes	+++	Heavy sedation
12	10.5	102	80	No	No	No	+	Sedation
14	13	80	68	Yes	Yes	Yes	+++	Depression, sleep
15	7	128	120	Yes	Yes	Yes	+++	Sedation
16	9.5	84	70	No	Yes	Yes	+	Sedation
17	8	152	100	No	No	No	++	Depression, sleep
19	9	120	80	No	Yes	Yes	+++	Sedation

TABLE 3

ACETYLCHOLINE CONTENT OF DIFFERENT TISSUES OF THE DOG AFTER INTRA-  
VENOUS ADMINISTRATION OF 0.75 MG OF RESERPINE

Results are expressed in  $\mu\text{g/g}$  of tissue. Probability of no difference between means of treated and untreated animals was calculated by the "t" test

No. of expt.	Weight of dog (kg)	Tissue		
		Hypothalamus	Ileum	Sino-atrial node
1	13	3.147	4.006	8.680
2	10	3.339	4.160	8.370
3	10.5	3.000	3.353	9.250
4	6	3.008	3.826	8.530
6	6.5	3.504	3.896	9.107
	Mean	3.199	3.848	8.787
	Standard deviation	$\pm 0.218$	$\pm 0.304$	$\pm 0.377$
	P	$> 0.8$	$> 0.1$	$< 0.05$
	Mean increase in acetylcholine content	6.7%	14.5%	17.9%

TABLE 4

SOME GENERAL EFFECTS OF INTRAVENOUS ADMINISTRATION OF 0.75 MG OF  
RESERPINE INTO DOGS

+ = Slight; ++ = moderate

No. of expt.	Weight of dog (kg)	Heart rate (beats/min)		Defaecation	Salivation	Emesis	Miosis	Behavioural changes
		Control	Minimum after reserpine					
1	13	90	92	No	No	No	+	Normal
2	10	88	74	No	No	No	No	Light sedation
3	10.5	104	94	No	No	No	+	Light sedation
4	6	130	114	No	No	No	++	Light sedation
6	6.5	124	120	No	No	No	+	Sedation ?

## DISCUSSION

The acetylcholine contents of the ileum and the sino-atrial node in control dogs were nearly the same as observed by Malhotra & Das (1962), but the content was lower in the hypothalamus; we cannot explain this low value, although it might be due to cerebral trauma produced by implantation of a Collison's cannula into the lateral ventricle.

The results show that reserpine increased the acetylcholine content of the central (hypothalamus) and the peripheral (ileum and sino-atrial node) tissues. The total level of acetylcholine in the brain is related to physiological changes in nervous activity such as sleep or wakefulness (Richter & Crossland, 1949). Feldberg (1957) observed that generally the content of acetylcholine in the brain increased during sedation, sleep and anaesthesia, while during convulsions it decreased. More recently, Giarman & Pepeu (1962) observed that in rats, depression of the central nervous system induced by a wide variety of drugs, with the notable exception of

reserpine, was generally associated with an elevation in the content of acetylcholine in the brain. With repeated administration of reserpine, there was an increase in the cerebral acetylcholine content after the first dose but a return to normal levels after subsequent doses, despite continued depression of the animals. In the present study, we could not find any quantitative correlation between the content of acetylcholine in the hypothalamus and the degree of sedation of the dogs. Dog No. 11 was very sedated and dog No. 17 slept, yet the increase in the acetylcholine content was small and less than the mean for the group; in dog No. 3, which showed only slight sedation following excitement, the increase in the acetylcholine content was quite large.

The peripheral effects of parasympathetic overactivity on pupillary size, heart rate and intestinal motility (miosis, bradycardia and defaecation) observed in some patients during treatment with reserpine (Vakil, 1954) and also seen experimentally in dogs after intravenous administration of reserpine (Malhotra & Das, 1962) were consistently observed in the present study. These peripheral effects of intracerebroventricular administration (Table 2) can without doubt be attributed to a central action of reserpine. Leakage of reserpine into the systemic circulation, if it occurred at all, can be excluded from responsibility since the total amount of reserpine injected into the cerebral ventricle was relatively small. Moreover, intravenous reserpine, in amounts equivalent to those given into the cerebral ventricle, did not produce any significant general effects. Also, with this amount of reserpine given intravenously, the increase in acetylcholine content was only 6.7%, 14.5% and 17.9% for the hypothalamus, the ileum and the sino-atrial node respectively, compared to 22.3%, 42.8% and 40.9% in these tissues after intracerebroventricular administration. Thomas (1957) observed salivation, defaecation and slight miosis after intracerebroventricular administration of reserpine. Reserpine has no direct peripheral parasympathomimetic action (Beckman, 1961). Bein (1956) showed that reserpine did not block transmission in autonomic ganglia. These findings also suggest that reserpine has a central action.

The increase in the acetylcholine content of the ileum and the sino-atrial node (42.8% and 40.9%) is statistically significant after intracerebroventricular administration of reserpine though the increase is not as large as reported previously (Malhotra & Das, 1962) to follow intravenous administration of 0.5 mg/kg of reserpine into the dog, the increase being 59.2% in the ileum and 75.3% in the sino-atrial node. This result may be due to the difference in doses of reserpine.

The present studies indicate that the peripheral parasympathetic effects as well as the increase in the acetylcholine content of the peripheral tissues are due mainly to a central action of reserpine.

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