Brit. J. Pharmacol. (1962), **18**, 190–193.

EFFECT OF RESERPINE ON THE ACETYLCHOLINE CONTENT OF THE HEART, THE ILEUM AND THE HYPOTHALAMUS OF THE DOG

BY

C. L. MALHOTRA AND P. K. DAS

From the Department of Pharmacology and Therapeutics, Lady Hardinge Medical College, New Delhi, India

(*Received November* 9, 1961)

The effect of intravenous administration of reserpine in 10 dogs was studied on the acetylcholine content of the sino-atrial node, the right auricle and the right ventricle of the heart, the ileum and the hypothalamus of dog. The general sedation, the bradycardia, and the purgative effects of reserpine were also noted. Tissues were removed for estimation of acetylcholine when bradycardia was maximal. Reserpine increased the acetylcholine content of all the tissues studied. The increase in the peripheral tissues was greater than in the hypothalamus. The bradycardia and the purgative effects of reserpine may be related to the increase of the acetylcholine content of the sino-atrial node and the ileum.

It has now been well established that reserpine depletes the brain and various peripheral tissues of the body of their 5-hydroxytryptamine and noradrenaline. Reserpine decreases the 5-hydroxytryptamine content of brain, especially of the hypothalamus (Pletscher, Shore & Brodie, 1956), the gastrointestinal tract (Pletscher, Shore & Brodie, 1955) and the platelets (Shore, Pletscher, Tomich, Kuntzman & Brodie, 1956); it also depletes the noradrenaline of the brain, particularly of the hypothalamus (Holzbauer & Vogt, 1956; Shore, Olin & Brodie, 1957), the adrenal gland (Holzbauer & Vogt, 1956; Taketomo, Shore, Tomich, Kuntzman & Brodie, 1957), the heart (Paasonen & Krayer, 1958), and the spleen and blood (Berger, 1956). Anand, Dua & Malhotra (1957) showed that reserpine not only depressed the sympathetic centres in the diencephalon but also facilitated the parasympathetic centres. Recently, Malhotra & Pundlik (1959) observed that reserpine increased the acetylcholine content of the frontal and temporal lobes, the cerebellum, the spinal cord and specially the hypothalamus of dog. This might be in some way related to the facilitation of parasympathetic centres in hypothalamus. The acetylcholine content of the hippocampus was, however, reduced. Reserpine is known to produce symptoms suggesting parasympathicotonia (Vakil, 1954; Doyle & Smirk, 1955). Since acetylcholine is known to be the peripheral parasympathetic transmitter, it was considered worth while to study the effect of reserpine on this neurohormone in some peripheral tissues of the dog, such as ileum and different areas of heart.

METHODS

Seventeen dogs have been used in this study; seven served as controls while ten received 0.5 mg/kg body weight of reserpine intravenously. The heart rate was recorded at intervals of 5 min after the administration of reserpine. The degree of sedation and passage of faeces, if any, were also noted. When the heart rate had reached its minimum and there was a tendency to rise again, the animals were anaesthetized with ether, and the skull, the abdomen and the chest were opened simultaneously. The following tissues were removed and immediately transferred to labelled weighing bottles that had been kept in a freezing mixture: (*a*) An annular piece of the proximal part of the ileum containing all layers, (*b*) hypothalamus, (*c*) the area of the sino-atrial node (upper two-thirds of the sulcus terminalis), (*d*) the right auricular appendix, and (*e*) a piece of anterior wall of the right cardiac ventricle. Acetylcholine was extracted from the tissues and assayed by the method of Nachmansohn, as described elsewhere (Anand, 1952). The control experiments were interspersed between the experiments with reserpine. The values of acetylcholine in the control animals were compared with those obtained with reserpine-treated dogs.

RESULTS

Acetylcholine contents of the hypothalamus, the ileum, the sino-atrial node, the right auricle and right ventricle of the heart of control dogs and dogs after administration of reserpine are given in Table 1. Table 1 shows that the acetylcholine

TABLE 1

ACETYLCHOLINE CONTENT OF DIFFERENT TISSUES OF DOG

(Results are expressed in μg/g tissue. Probability of no difference between means of treated and untreated animals calculated by " t " test)

Expt. no.	Wt in kg and sex of dog	Time of removal of tissue	Hypothalamus	Ileum	Heart		
					S.A. node	Right auricle	Right ventricle
Control							
1	7·0 M		4·92	3·48	6·80	2·76	0·65
3	6·0 F		5·36	3·42	9·01	4·98	1·15
6	8·0 F		3·76	4·18	5·89	2·26	0·66
9	5·2 M		5·00	4·00	6·59	2·69	0·52
12	12·0 M		5·00	2·00	5·62	2·50	0·62
15	8·5 F		4·71	3·22	6·86	3·17	0·80
16	6·5 F		4·91	3·58	6·56	2·91	0·64
Mean ±s.d.			4·81 ±0·501	3·41 ±0·707	6·76 ±1·095	3·04 ±0·903	0·72 ±0·207
Treated with reserpine							
2	6·5 M	2 hr.	5·61	4·49	5·55	3·41	0·87
4	5·5 F	1½ hr.	6·08	4·76	11·33	5·66	2·27
5	13·0 F	1 hr.	6·37	7·13	8·60	4·06	1·00
7	9·5 F	¾ hr.	6·00	4·90	16·31	6·18	0·63
8	9·5 M	½ hr.	5·32	6·56	13·46	4·00	0·88
10	13·5 M	¾ hr.	4·35	5·66	6·47	3·91	0·89
11	5·0 M	½ hr.	8·28	5·66	20·72	5·71	1·49
13	5·5 M	½ hr.	5·17	4·59	22·37	4·76	0·79
14	6·5 F	½ hr.	6·84	6·04	4·95	3·71	0·88
17	8·5 M	½ hr.	6·05	4·52	8·71	3·67	0·68
Mean ±s.d.			6·01 ±1·058	5·43 ±0·930	11·85 ±6·225	4·51 ±1·000	1·04 ±0·492
P			<0·05	<0·01	<0·05	<0·01	>0·05
Mean increase in acetylcholine content			24·9%	59·2%	75·3%	48·3%	44·4%

content of different tissues in control dogs varied widely. Even in the heart itself the acetylcholine contents of the sino-atrial node and auricle were approximately nine and four times that of ventricle. Reserpine was found to increase the acetylcholine content of all the tissues studied. The difference between the level of acetylcholine content of ventricle in the control and treated groups was, however, not statistically significant. Reserpine generally increased the acetylcholine level of the peripheral tissues more (44.4% to 75.3%) than that of hypothalamus (24.9%).

The bradycardia and the purgative and gross behavioural effects of reserpine have been summarized in Table 2.

TABLE 2

SOME GENERAL EFFECTS OF RESERPINE IN DOG

* There was transient initial tachycardia

Expt. no.	Effect on heart rate/min			Passage of faeces		Behaviour effects
	Initial	Minimum after reserpine	Time (in min) when minimum reached	Yes/No	Time (in min) when passed	
2	146	64	30	Yes	60 & 90	Depressed, sleeping
4	100	84	60	Yes	15	Sedated
5	114	92	40	Yes	30 & 45	Excitement followed by sedation
7	116	90	30	No		Sedated
8	90	60	20	Yes	15	Excitement followed by sedation
10	86	70	30	No		Depressed, sleeping
11	162	140	10	No		Sedated
13	102	102*	20	Yes	10	Sedated
14	170	144	20	No		Depressed, sleeping
17	180	170*	20	Yes	10	Depressed, sleeping

DISCUSSION

The results show that reserpine increased the acetylcholine content of some peripheral tissues, such as ileum and different regions of the heart. It has already been shown by Malhotra & Pundlik (1959) that reserpine increased the acetylcholine content of some areas of the brain. It appears that the effect of reserpine is of a generalized nature on the different neurohormones of the tissues. While there occurs a depletion of 5-hydroxytryptamine and the catechol amines, there is an increase of acetylcholine in central and peripheral tissues. The bradycardia and the purgative effects of reserpine may be related to the increased acetylcholine content of heart and intestine. It was interesting to note that reserpine caused a much greater increase in the acetylcholine of the heart and the ileum than that of the hypothalamus. In the heart itself the increase was greater in the region of the pacemaker (75.3%) than in the auricle (48.3%) and ventricle (44.4%). Some of the symptoms, such as bradycardia, increased intestinal motility, miosis, aggravation of existing bronchial asthma, renal colic and biliary colic, observed in some patients under treatment with reserpine (Vakil, 1954 ; Doyle & Smirk, 1955) do not appear to be only the result of a passive, relative increase of parasympathetic activity due

to depletion of sympathetic transmitters but are probably due to an active facilitation of the parasympathetic system—both centrally (Anand, Dua & Malhotra, 1957; Malhotra & Pundlik, 1959) and peripherally. Ludany, Gati & Hideg (1958) have, however, reported that increased motility of intestinal villi after giving reserpine is not affected by vagotomy but blocked by atropine, suggesting a peripheral action of reserpine.

REFERENCES

ANAND, B. K. (1952). Influence of temperature on vagal inhibition and liberation of acetylcholine in frog heart. *Amer. J. Physiol.*, **168**, 218–225.

ANAND, B. K., DUA, S. & MALHOTRA, C. L. (1957). Effects of reserpine on blood-pressure responses evoked from the hypothalamus. *Brit. J. Pharmacol.*, **12**, 8–11.

BERGER, M. (1956). Abnahme der Plasma-Nor-adrenalin-Konzentration nach Reserpin. *Helv. Physiol. Pharm. Acta*, **14**, C13–C14.

DOYLE, A. E. & SMIRK, F. H. (1955). Drug therapy in hypertension; critical review. *Practitioner*, **174**, 135–143.

HOLZBAUER, M. & VOGT, M. (1956). Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. *J. Neurochem.*, **1**, 8–11.

LUDANY, G., GATI, T. & HIDEG, J. (1958). Reserpin und die Darmzottenbewegung. *Arch. int. Pharmacodyn.*, **114**, 227–231.

MALHOTRA, C. L. & PUNDLIK, P. G. (1959). The effect of reserpine on the acetylcholine content of different areas of the central nervous system of the dog. *Brit. J. Pharmacol.*, **14**, 46–47.

PAASONEN, M. K. & KRAYER, O. (1958). The release of norepinephrine from the mammalian heart by reserpine. *J. Pharmacol. & exp. Therap.*, **123**, 153.

PLETSCHER, A., SHORE, P. A. & BRODIE, B. B. (1955). Evidence that serotonin has role in brain function. *Science*, **122**, 968.

PLETSCHER, A., SHORE, P. A. & BRODIE, B. B. (1956). Serotonin as a mediator of reserpine action in brain. *J. Pharmacol. & exp. Therap.*, **116**, 84–89.

SHORE, P. A., OLIN, J. S. & BRODIE, B. B. (1957). Release of brain norepinephrine by reserpine. *Fed. Proc.*, **16**, 335–336.

SHORE, P. A., PLETSCHER, A., TOMICH, E. G., KUNTZMAN, R. & BRODIE, B. B. (1956). Release of blood platelet serotonin by reserpine and lack of effect on bleeding time. *J. Pharmacol. & exp. Therap.*, **117**, 232–236.

TAKETOMO, Y., SHORE, P. A., TOMICH, E. G., KUNTZMAN, R. & BRODIE, B. B. (1957). Studies on the mechanism of reserpine-induced epinephrine release and hyperglycemia. *J. Pharmacol. & exp. Therap.*, **119**, 188.

VAKIL, R. J. (1954). Rauwolfia serpentina in the treatment of high blood-pressure. *Lancet*, **ii**, 726.