Brit. J. Pharmacol. (1959), 14, 46.

THE EFFECT OF RESERPINE ON THE ACETYLCHOLINE CONTENT OF DIFFERENT AREAS OF THE CENTRAL NERVOUS SYSTEM OF THE DOG

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(RECEIVED OCTOBER 3, 1958)

The acetylcholine content of the frontal and temporal lobes, hippocampus, hypothalamus, cerebellum and spinal cord was estimated in 18 dogs 3 hr. after the intravenous administration of reserpine. Another group of 7 dogs served as control. After reserpine (0.5 mg./kg. body weight) there was an increase of acetylcholine in all the areas under study except in the hippocampus. The increase was specially marked in the hypothalamus. In the hippocampus, however, reserpine caused a significant decrease of acetylcholine.

In spite of the large amount of experimental work done with reserpine, the mechanism of its action still remains obscure. Anand, Dua, and Malhotra (1957) showed by implanting electrodes in the hypothalamus that reserpine not only depressed the sympathetic centres in the diencephalon, but also facilitated the parasympathetic centres. Holzbauer and Vogt (1956) found that the noradrenaline concentration in the hypothalamus of the cat was reduced by a single injection of reserpine. Pletscher, Shore, and Brodie (1956) observed that reserpine caused a severe loss of 5-hydroxytryptamine from brain tissue, especially from the hypothalamus. Brodie and Shore (1957) attributed the action of reserpine to the release of 5-hydroxytryptamine in the free form from depots in the brain and also considered it to be a possible central parasympathetic transmitter. It was considered worth while to study the effect of reserpine on the acetylcholine content of different areas of central nervous system as no work had been done on this subject and because acetylcholine has already been established beyond doubt to be the chemical transmitter of nerve impulses at some sites in the peripheral autonomic nervous system.

METHODS

Twenty-five dogs were used. Seven served as controls and the remaining eighteen received 0.5 mg./kg. body weight of reserpine intravenously. The animals were anaesthetized with ether, the skull opened and the whole brain removed as quickly as possible 3 hr. after the dog had received reserpine. It was immediately transferred to a beaker which had been kept in a freezing mixture. With the beaker still in the mixture the following portions of the brain were removed with scissors and placed in labelled weighing bottles that had also been kept in freezing mixture: (a) The anterior portion of the frontal lobe, (b) part of the temporal lobe including the periamygdaloid region, (c) part of the hippocampus, (d) the entire hypothalamus, (e) part of the cerebellar grey matter, and (f) part of the cervical segments of the spinal cord.

Acetylcholine was extracted from these portions of the central nervous system and assayed by the method of Nachmansohn described elsewhere (Anand, 1952). A control experiment was usually placed between two or three experiments with reserpine. Estimates of acetylcholine in the control animals were compared with those obtained treated with reserpine.

RESULTS

The estimates of acetylcholine in control dogs and dogs which had received reserpine are given in Table I. It will be seen that acetylcholine was widely though unevenly distributed in the central nervous system of the control group, the amount being highest in the hypothalamus and lowest in the cerebellum. The areas comprising the limbic system, the so-called " visceral brain," namely the hippocampus, that portion of the temporal lobe which includes the periamygdaloid region and the anterior portion of the frontal lobe, contained appreciable amounts of acetylcholine though less than the hypothalamus. On comparing the average values of acetylcholine in the dogs which received reserpine with those obtained from controls, it is clear that the administration of reserpine resulted in a significant increase in the

TABLE I

ACETYLCHOLINE CONTENT OF DIFFERENT AREAS OF DOG CENTRAL NERVOUS SYSTEM

Results are expressed in µg./g. tissue. Probability of differences between means of treated and untreated animals being due to chance also shown at the foot of the table.

Dog No.	Hypo-thalamus	Hippo-campus	Tempo-ral Lobe	Frontal Lobe	Cere-bellum	Spinal Cord
Control						
1	2·80	2·65	2·20	2·13	1·05	1·30
2	3·70	2·47	2·63	2·32	1·09	1·07
3	3·60	3·60	2·57	1·38	1·04	1·75
4	3·35	3·03	2·82	1·22	0·93	1·15
5	2·86	3·25	2·17	1·40	1·00	1·47
6	3·69	2·85	2·05	1·50	0·88	1·73
7	3·75	3·60	2·00	1·90	1·02	1·24
Mean ± s.d.	3·39 ±0·406	3·06 ±0·443	2·43 ±0·307	1·69 ±0·422	1·00 ±0·025	1·39 ±0·271
Treated						
1	3·71	2·52	2·17	2·14	1·27	1·72
2	3·76	2·38	2·95	2·46	1·35	1·55
3	4·27	1·98	2·94	3·21	0·95	1·45
4	4·39	2·17	2·82	1·76	1·20	1·72
5	5·56	2·94	3·05	4·27	1·10	1·58
6	5·72	4·62	3·52	3·80	2·35	3·05
7	3·60	1·72	2·62	1·93	1·05	1·57
8	2·99	1·28	2·71	1·75	1·25	1·95
9	3·67	1·80	2·92	2·52	1·44	1·72
10	2·98	1·98	2·06	1·56	1·15	1·51
11	5·45	2·60	3·80	2·54	1·28	1·59
12	4·58	1·96	2·55	1·98	1·45	1·95
13	3·80	2·25	2·77	2·25	1·04	1·75
14	3·45	1·85	2·22	2·00	1·06	1·84
15	5·56	2·44	3·22	1·87	1·37	1·43
16	4·43	1·98	2·85	2·28	1·15	1·56
17	4·55	2·13	3·15	2·22	1·27	1·75
18	3·99	1·42	2·90	1·99	1·39	1·58
Mean ± s.d.	4·25 ±0·866	2·22 ±0.725	2·85 ±0·440	2·36 ±0·718	1·28 ±0·302	1·74 ±0·361
P	<0·02	<0·01	<0·05	<0·05	<0·05	<0·05

acetylcholine content of all areas of the brain under study except hippocampus, the acetylcholine content of which actually decreased.

The observation that reserpine caused a decrease in the acetylcholine content of the hippocampus is quite significant and interesting. In this connexion, Anand, Malhotra, Singh, Pundlik, and Chhina (1958) found that although hypothermia caused a decrease in the acetylcholine content of the hypothalamus, the frontal and temporal lobes of the brain and of the heart, it increased the acetylcholine content of the hippocampus. This area of the brain seems to be affected in a direction opposite from that of the other areas as far as its metabolic pattern is concerned. The morphological appearance and the biochemical constitution of the hippocampus has been investigated by Lowry, Roberts, Leiner, Wo, Fan, and Albens (1954). It has been pointed out that it differs from other regions of the cortical surface, such as the frontal and occipital lobes, in several respects.

Kim and Maclean (1956), while comparing the effects of reserpine on the electrical responses of the limbic and neocortical structures, found that about 4 hr. after the administration of reserpine the recordings from the hippocampus began to show much rhythmic activity at a frequency of 3.5 to 4 cycles/sec. This activity was conspicuous only in the waking state during which the neocortex showed desynchronization. The contrast between the electrical activities of the hippocampus and neocortex becomes even more marked 20 hr. after giving reserpine. The available evidence therefore suggests an important inverse relationship between the hippocampus and other areas of the brain as far as their metabolic patterns are concerned, and the hippocampus may play an important role in autonomic function.

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