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REVERSAL BY 3,4-DIHYDROXYPHENYLALANINE OF RESERPINE-INDUCED REGIONAL CHANGES IN ACETYLCHOLINE CONTENT IN GUINEA-PIG BRAIN

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Abstract—An investigation was made on the effect of reserpine, both alone and combined with DOPA and Iproniazid, on the ACh content in different cerebral areas of unanaesthetized guinea-pigs.

Reserpine lowers ACh levels in the cerebral cortex only to some degree; in the caudate nucleus the fall is marked.

DOPA, which does not change the neurotransmitter content, reverses the reserpine effect.

Iproniazid reduces ACh content in the thalamus. If reserpine is given in Iproniazid-treated animals, an evident reduction is detectable in the brain stem, thalamus and hypothalamus, but not in caudate nucleus.

The above results show that the effect of reserpine, on the cholinergic telencephalic structures is reversed or prevented by refilling or rising dopamine levels.

On the other hand, the alerting state of the animal as caused by Iproniazid plus reserpine, seems to be associated with the decrease of ACh in the rhombomesodiencephalic areas.

A SIGNIFICANT increase of acetylcholine (ACh) in some cerebral areas, with the exception of hippocampus, was found by Malhotra and Pundlik[1] in reserpine-treated dogs under ether anaesthesia. Such findings were subsequently confirmed in the entire brain of unanaesthetized rats[2].

The present study was undertaken to establish the regional changes in brain ACh in unanaesthetized, reserpine-treated animals, because the use of an anaesthetic agent or the ACh assay in the entire brain could have masked some interesting zonal effects of the drug. Furthermore, (i) choline acetyltransferase activity was determined and (ii) the effect of 3,4-dihydroxyphenylalanine (DOPA) and Iproniazid on normal and reserpine-treated animals was tested.

EXPERIMENTAL

1. Guinea-pigs of both sexes weighing 250–350 g were used; one group was treated with a single dose of reserpine (2·5 mg/kg, i.p.), and the other received 0·25 mg/kg for 7 days, i.p. The animals of the first group were kept either at room temperature (about 20°) or at 28° and killed 2,4 und 24 hr after injecting the drug. The animals treated for 7 days were kept at room temperature and killed 24 hr after the last injection.

2. When testing the effects of DOPA and Iproniazid, the drugs (200 mg/kg, i.p.) were given respectively 30 min and 24 hr before killing, both in normal and reserpine-treated animals (2·5 mg/kg, i.p.).

The following brain areas were chosen: (1) a parasagittalis frontoparietal slice of cerebral cortex (CC); (2) caudate nucleus (CN); (3) thalamus (Th); (4) hypothalamus (Hypth) and (5) the ventromedialis part of the rhombomesencephalic brain stem (BS).

Total tissue ACh was extracted with the method of Beani and Bianchi;[3] choline acetyltransferase activity was determined at 37° according to the method of Bull *et al.*[4]

The samples were biologically assayed on eserinized frog's rectus abdominis muscle; with the aim to exclude any interference of the drugs present in the brain extracts, standards were prepared by adding known ACh amounts to a part of the samples previously boiled at pH 10.

In each experiment the brains of 1 control and 1–2 treated animals were jointly examined to compensate for the spontaneous variations in neurotransmitter levels.

Reserpine was dissolved in 2×10^{-2}M H_3PO_4 (0·1 %, w/v) and DOPA in 1 N HCl (5 %, w/v), immediately before use. Both solutions were brought to pH 5 with 1 N NaOH.

Reserpine, kindly supplied by CIBA, d-l DOPA BDH (A grade) and Iproniazid Roche were used.

In the text, the amounts of ACh are given as chloride.

RESULTS

The effect of reserpine

After the single dose of reserpine (2·5 mg/kg) the usual symptoms were present at the time of killing.

In preliminary experiments, no difference had been found in the ACh content of the entire cerebrum (midbrain plus forebrain), between controls and animals treated with reserpine 24 hr earlier: in fact, the values obtained were: $3{\cdot}77 \pm 0{\cdot}38$ and $3{\cdot}75 \pm 0{\cdot}33$ μg/g ± S.D. of fresh tissue, respectively (seven experiments).

When a zonal study was carried out (Table 1), a significant fall in the ACh levels was detected in the CN,4 and 24 hr after treatment. The ACh level also dropped in the CC after 4 hr, but it approached the control values after 24 hr. No significant changes occurred in the Th, Hypth and BS.

When the animals were kept at 28° and killed 4 hr after the drug, the symptoms of reserpine treatment and the ACh depletion in the CC and CN were still clearly evident (Table 2).

The choline acetyltransferase activity in the CC and CN was unaffected 24 hr after drug (Table 3).

At the end of subacute treatment the behaviour of the animals was nearly normal: only a certain degree of eyelid ptosis and a fall in body weight were observed. At this point, no significant changes in ACh content were detected (Table 1).

The effect of DOPA

In normal animals no gross changes in behaviour and ACh levels occurred within 30 min after DOPA injection. A slight and insignificant increase in ACh occurred only in the BS, (values not given). Guinea-pigs pretreated with reserpine 2·5 mg/kg 4 hr earlier, resumed normal behaviour or else showed signs of over-activity 30 min

TABLE 1. EFFECT OF RESERPINE ON ACETYLCHOLINE LEVELS IN DIFFERENT AREAS OF GUINEA-PIG BRAIN

Area	Controls (34)	Acute treatment, reserpine 2·5 mg/kg, i.p.			Subacute treatment, reserpine 0·25 mg/kg, i.p., 7 days.	
		After 2 hr (13)	After 4 hr (15)	After 24 hr (12)	Controls (10)	24 hr after the 7th dose (9)
Cerebral cortex	3·18 ± 0·78	2·93 ± 0·63	2·60 ± 0·60*	2·90 ± 0·53	3·40 ± 0·58	3·67 ± 0·48
Caudate nucleus	5·51 ± 1·21	5·35 ± 0·90	4·10 ± 0·71‡	4·40 ± 0·92†	5·28 ± 1·00	5·61 ± 1·20
Thalamus	5·76 ± 1·07	6·11 ± 0·88	5·57 ± 0·87	5·30 ± 0·40	5·60 ± 0·39	5·36 ± 0·84
Hypothalamus	4·50 ± 1·18	4·30 ± 0·84	4·12 ± 0·65	4·13 ± 0·65	4·71 ± 0·86	4·37 ± 0·79
Brain Stem	5·67 ± 1·04	6·07 ± 0·68	5·81 ± 0·77	5·11 ± 0·80	5·29 ± 0·74	5·02 ± 1·07

* $< P$ 0·02
† $< P$ 0·01
‡ $< P$ 0·001
The results are expressed in ACh μg per g wet tissue ± S.D.; in brackets, the number of experiments.

TABLE 2. EFFECT OF DOPA, RESERPINE AND RESERPINE PLUS DOPA ON ACETYLCHOLINE LEVELS IN CEREBRAL CORTEX AND CAUDATE NUCLEUS OF GUINEA-PIG BRAIN

Area	Controls(34)	DOPA 200 mg/kg 30 min (8)	Reserpine 2·5 mg/kg, 4 hr, 20° (13)	Reserpine 2·5 mg/kg, 4 hr, 28° (6)	Reserpine 2·5 mg/kg, 4 hrs DOPA 200 mg/kg, 30 min, 20° (10)
Cerebral cortex	3·18 ± 0·78	3·08 ± 0·43	2·53 ± 0·18*	2·53 ± 0·31*	2·95 ± 0·26
Caudate nucleus	5·51 ± 1·21	5·50 ± 0·93	3·78 ± 0·65†	3·26 ± 0·46†	4·91 ± 0·80

* $P < 0·02$
† $P < 0·001$
The results are expressed in ACh μg per g wet tissue ± S.D.; in brackets, the number of experiments.

after the same dose of DOPA.[5] At this time the ACh levels in the CC and CN were close to those of controls (Table 2).

The effect of Iproniazid

A mild excitation was present in guinea-pigs 24 hr after Iproniazid injection.

ACh amounts were normal in the CC and CN, but lower in the Hypth, and BS and particularly low in the Th, where the level of statistical significance was reached.

TABLE 3. EFFECT OF RESERPINE ON CHOLINE ACETYLTRANSFERASE ACTIVITY IN CEREBRAL CORTEX AND CAUDATE NUCLEUS OF GUINEA-PIG BRAIN

Area	Control	Reserpine 2·5 mg/kg, 24 hr
Cerebral cortex	1136 ± 90(6)	1123 ± 154(6)
Caudate nucleus	5623 ± 1309(13)	5650 ± 1390(13)

The results are expressed in μg of ACh formed per g wet tissue per hr ± S.D. In brackets, the number of experiments.

TABLE 4. EFFECT OF IPRONIAZID AND IPRONIAZID PLUS RESERPINE ON ACETYLCHOLINE LEVELS IN DIFFERENT AREAS OF GUINEA-PIG BRAIN

Area	Controls (12)	Iproniazid 200 mg/kg 24 hr (9)	Iproniazid 200 mg/kg, 24 hr plus reserpine 2·5 mg/kg, 4 hr (12)
Cerebral cortex	2·65 ± 0·54	2·57 ± 0·44	2·64 ± 0·36
Caudate nucleus	4·97 ± 0·70	5·13 ± 0·65	4·70 ± 0·69
Thalamus	5·02 ± 0·42	4·49 ± 0·40†	4·01 ± 0·60§
Hypothalamus	3·42 ± 0·81	3·26 ± 0·75	2·86 ± 0·36*
Brain stem	5·29 ± 0·70	4·73 ± 0·67	4·39 ± 0·65‡

* $P<0·05$ † $P<0·02$ ‡ $P<0·01$ § $P<0·001$

The results are expressed in ACh μg per g wet tissue ± S.D.; in brackets, the number of experiments.

When reserpine was given 4 hr before killing, a strong excitation and signs of sympathetic stimulation developed, as previously described for other rodents.[6, 7]

In this group, there was no change in the CC and CN, but a marked, significant reduction was detected in the Th, Hypth and BS (Table 4).

DISCUSSION

The difference between our findings and those of Malhotra and Pundlik[1] and Giarman and Pepeu[2] may be due to the different species of animals employed and to the experimental design. Ether anaesthesia probably plays an important rôle because: (i) anaesthetic agents per se increase the ACh content in the brain and (ii) reserpine potentiates anaesthesia.

Our results show that the drug, in one single dose, reduces ACh content:

(*a*) in the CC and CN of normal guinea-pigs, even kept at 28°, when hypothermia is prevented;

(*b*) in the Th, Hypth and BS after pretreatment with Iproniazid, which, when given alone, is capable of significantly depleting ACh content only in the TH.

The effect of reserpine, is, therefore, restricted to different cholinergic structures, under the particular experimental condition (normal or IMAO pretreated animals). This fact argues against the hypothesis of a direct interference by the drug in cholinergic neurones in general.[8]

Furthermore, the ineffectiveness of reserpine to change ACh release and stores in the cholinergic motor nerve terminals,[9] and to alter cholineacetyltransferase activity, excludes any direct action on ACh synthesis, storage and release.

Even if the intimate mechanism of ACh depletion remains to be explained, this probably depends on the changes produced by the drug on noncholinergic neurones, which, in turn, modify the functional state of the cholinergic ones.

The effect of reserpine on normal animals seems to follow the exhaustion of dopamine stores, because ACh depletion is evident, above all, in the CN and it is reverted 30 min after DOPA administration. At this point, dopamine stores are the only ones refilled by DOPA.[10] The supposed dopaminergic neurones[11, 12] seem therefore involved in modulating the functional state of the telencephalic cholinergic structures.[13] In this respect the normal amounts of available dopamine have probably a ceiling effect, because higher levels obtained either through DOPA,[14] or IMAO administration[15] do not change ACh content in the CC and CN. The absence of gross behavioural signs and of changes in ACh content[2] in 7-day reserpine-treated animals, is in agreement with the suggestion of cellular adaptation to low levels of monoamine, during long-term treatment.[16]

Some difficulties arise when explaining the depletion of ACh produced by Iproniazid and above all, by Iproniazid plus reserpine in the Th, Hypth and BS

The complex mechanism of IMAO does not allow us to infer that ACh depletion depends solely on the increased levels of Noradrenaline and 5-hydroxytriptamine.[17] Furthermore, the effect is particularly prominent when Iproniazid is followed by reserpine: it is well known that, in the latter case, there is no change in the increased cerebral amine levels, provoked by IMAO alone, but there are, on the other hand, dramatic consequences in the behaviour of the animals.[6, 7] The only conclusion, therefore, to be reached is that the activation of neuronic pools governing the alerting state, is coupled with substantial changes in the cholinergic structures in the Th, Hypth and BS.

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