Brit. J. Pharmacol. (1962), **19**, 153–160.

DRUG-INDUCED CHANGES IN THE CONCENTRATION OF 5-OR INDOLYL COMPOUNDS IN CEREBRO-SPINAL FLUID AND CAUDATE NUCLEUS

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(*Received April* 26, 1962)

The effects of reserpine and imipramine on the concentration of indolyl compounds bearing an OR group in position 5 (5-OR indolyl compounds) in the cerebrospinal fluid and the caudate nucleus of the dog have been studied. Reserpine was shown to produce an increase in the concentration of these compounds in the cerebrospinal fluid. In the caudate nucleus reserpine produced a fall in the concentration of the basic 5-OR indolyl compounds accompanied by an equivalent increase in the concentration of acidic 5-OR indolyl compounds. No significant change in the concentration of 5-OR indolyl compounds in the cerebrospinal fluid was observed after treatment with imipramine.

In a previous publication (Ashcroft & Sharman, 1960) it was reported that there was a difference in the concentration of 5-hydroxyindolyl compounds in the cerebrospinal fluid of patients suffering from depressive psychoses and patients with neurological diseases. The present investigation on dogs was carried out to test the possibility that changes in the metabolism of 5-hydroxytryptamine in the body might be reflected in changes in the cerebrospinal fluid level of indolyl compounds bearing an OR group in position 5. Sharman (1960) has shown that many indolyl compounds bearing an OR group in position 5, where R is a hydrogen atom, or an aryl or alkyl group, and unsubstituted in the 2 position of the indole nucleus, show the fluorescence in 3N hydrochloric acid attributed to 5-hydroxyindolyl compounds by Udenfriend, Bogdanski & Weissbach (1955) and demonstrated for *N*-acetyl-5-methoxytryptamine by Axelrod & Weissbach (1961). It is possible that traces of indolyl compounds bearing an OR group in position 5 other than 5-hydroxytryptamine or 5-hydroxyindol-3-ylacetic acid are present in the cerebrospinal fluid. Because of this it was decided to refer to the substances estimated in this investigation as 5-OR indolyl compounds.

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METHODS

All-glass apparatus was used for the chemical procedures. It was found that the use of rubber stoppers led to interference with the fluorescence estimations. De-ionized water was used throughout. Samples of cerebrospinal fluid (approximately 2.5 ml.) were collected into glass-stoppered tubes, each containing 5 mg ascorbic acid to prevent oxidation.

Estimation of the total 5-OR indolyl compounds in cerebrospinal fluid

A measured portion (2.0 to 2.5 ml.) of cerebrospinal fluid was placed in a glass-stoppered, graduated test-tube. To the sample was added 0.5 ml. of a 10% (w/v) solution of zinc sulphate (Analar grade, recrystallized from water and dried) and the contents of the tube mixed by inversion. After the addition of 0.1 ml. of a 10% (w/v) solution of sodium hydroxide the contents of the tube were again mixed by inversion. To 2.0 ml. of the clear supernatant fluid, obtained after centrifuging for 10 min at 2,500 r.p.m., was added 1.0 ml. of concentrated hydrochloric acid containing 0.05% (w/v) ascorbic acid. The fluorescence of this solution at a wavelength of 550 mμ, when activated with ultra-violet light of wavelength 295 mμ, was measured in an Aminco-Bowman spectrophotofluorometer.

Estimation of 5-OR indolyl compounds in brain tissue

(a) Total 5-OR indolyl compounds. The weighed tissue was homogenized in 0.1 N hydrochloric acid (2 ml./g tissue). The homogenate was then diluted to 10 ml./g tissue with water. A 5 ml. portion of the diluted homogenate was deproteinized using 1.0 ml. of a 10% solution of zinc sulphate and 0.2 ml. of a 10% solution of sodium hydroxide in a manner similar to that described for cerebrospinal fluid ; 1.0 ml. of concentrated hydrochloric acid containing ascorbic acid was added to 2.0 ml. of the clear supernatant obtained after centrifuging at 2,500 r.p.m. for 10 min. The fluorescence of this solution (activation wavelength 295 mμ ; fluorescence wavelength 550 mμ) was measured.

(b) Basic 5-OR indolyl compounds. A portion of the supernatant fluid obtained after the protein precipitation was extracted into butanol and estimated fluorimetrically by the method described for 5-hydroxytryptamine by Bogdanski, Pletscher, Brodie & Udenfriend (1956).

(c) Acidic 5-OR indolyl compounds. These were estimated by a modification of the method described for 5-hydroxyindol-3-ylacetic acid by Udenfriend, Titus & Weissbach (1955). A 2.0 ml. portion of the clear supernatant fluid obtained after protein precipitation was adjusted to a *p*H of between 1 and 2 (indicator paper) with concentrated hydrochloric acid. The solution was saturated with sodium chloride and then shaken for 1 min with 10 ml. of diethyl ether, which had been stored previously over a saturated aqueous solution of sodium sulphite.

The two phases were separated completely by centrifuging for 30 sec and the ether layer was transferred to a tube containing 1.0 ml. of 0.5 M sodium phosphate buffer *p*H 7.0. The mixture was shaken for 1 min and then the two phases were allowed to separate. A measured portion (0.8 ml.) of the aqueous layer was diluted to 1.0 ml. with water and 0.5 ml. of concentrated hydrochloric acid containing ascorbic acid was added. The fluorescence of this solution (activation wavelength 295 mμ, fluorescence wavelength 550 mμ) was measured. The fluorescence in the estimation of total, basic and acidic 5-OR indolyl compounds was measured against and expressed as ng of 5-hydroxyindol-3-ylacetic acid.

Collection of the cerebrospinal fluid

Three procedures were employed for the collection of cerebrospinal fluid.

(1) Adult dogs were anaesthetized with ether followed by intravenous chloralose (70 mg/kg body weight). The head of the animal was securely fixed to allow the percutaneous introduction of a 19-gauge hypodermic needle into the cisterna magna. The needle was then secured in a rigid clamp. A narrow-bore manometer, into which some of the cerebrospinal fluid was allowed to escape, was connected to the needle via a three-way tap and gave a record of the pressure. The pressure, in mm cerebrospinal fluid, was read every 15 min. A first 2.0 to

2.5 ml. sample of cerebrospinal fluid was collected by allowing the fluid to drip from the tap into the collection tube ; further samples were only taken when the cerebrospinal fluid pressure had recovered from the fall due to sampling and had reached about 70 mm cerebrospinal fluid. This was usually a little below the initial cerebrospinal fluid pressure. In some cases the cerebrospinal fluid pressure was slow in recovering to this value, and it was found that the intravenous injection of 0.9% w/v saline accelerated recovery.

(2) A 19-gauge hypodermic needle was introduced into the cisterna magna of an anaesthetized dog as described in the preceding section.

Two ml. of cerebrospinal fluid was allowed to drip from the needle. Drugs were then injected intravenously and 2 hr later as much cerebrospinal fluid as possible was drained off in consecutive 2.0 ml. samples.

(3) A third procedure was adopted in order to examine the chronic effect of imipramine hydrochloride on the concentration of 5-OR indolyl compounds in the cerebrospinal fluid and to study the day-to-day variation in normal dogs.

Adult dogs were anaesthetized by an intravenous injection of thiopentone. A 19-gauge hypodermic needle was introduced percutaneously into the cisterna magna and 2 ml. of cerebrospinal fluid was withdrawn into a syringe. It was possible to repeat this procedure once daily, taking aseptic precautions throughout.

Collection of brain tissue

At the end of each experiment the dog was bled out and the brain removed. The caudate nuclei were dissected out, frozen and stored at −17° C until analysed. The caudate nucleus was selected because this tissue has a high content of 5-OR indolyl compounds and the dissection is easily reproducible.

RESULTS

The estimates of total 5-OR indolyl compounds in the cerebrospinal fluid of normal dogs and the effect produced by an intravenous injection of reserpine (2 mg/kg body weight) are shown in Table 1. They were obtained using the first method of cerebrospinal fluid collection. The values indicate that the increase in the cerebrospinal fluid concentration of 5-OR indolyl compounds after an intravenous injection of reserpine was greater than the increase observed to occur in successive samples taken from control animals. There was, however, no common time-course to these experiments. The time intervals between the collection of samples varied between 30 and 120 min. The second method of cerebrospinal fluid collection was introduced to standardize the timing in each experiment and to attempt to show more clearly whether there was an increase of 5-OR indolyl compounds in the cerebro-

TABLE 1

EFFECT OF RESERPINE ON THE CONCENTRATION OF TOTAL 5-OR INDOLYL COMPOUNDS IN THE CEREBROSPINAL FLUID (a)

(Cerebrospinal fluid collected by method 1)

Concentrations expressed as ng 5-hydroxyindol-3-ylacetic acid/ml. cerebrospinal fluid

		Concentration of total 5-OR indolyl compounds in cerebrospinal fluid					
Cerebrospinal fluid sample no.:		1	2	3	4	5	6
Control dogs	1	46	92	85	94	83	—
	2	59	71	83	96	94	92
	3	36	38	74	125	102	—
Reserpine (2 mg/kg) injected intravenously after sample 2	1	49	87	72	108	168	161
	2	33	71	135	197	—	—

TABLE 2

EFFECT OF RESERPINE ON THE CONCENTRATION OF TOTAL 5-OR INDOLYL COMPOUNDS IN THE CEREBROSPINAL FLUID (b)

(Cerebrospinal fluid collected by method 2)

Concentrations expressed as ng 5-hydroxyindol-3-ylacetic acid/ml. cerebrospinal fluid

	Concentration of total 5-OR indolyl compounds in cerebrospinal fluid				Concentration of total 5-OR indolyl compounds in caudate nucleus (ng/g)
	Initial conc.	Conc. in consecutive samples taken 2 hr after initial sample			
		1	2	3	
Control dogs	66	61	43	55	775
	45	48	49	55	705
Reserpine (2 mg/kg) intravenously after initial sample	34	70	102	127	810
	66	72	88	145	725
	54	101	125	147	805

spinal fluid after reserpine. The results obtained with this procedure are shown in Table 2. They show that 2 hr after a dose of reserpine the concentration of 5-OR indolyl compounds in the cerebrospinal fluid of 3 dogs had risen to approximately twice the concentration observed in control animals. It is also shown that the level of the 5-OR indolyl compounds in the caudate nucleus had not fallen 2 hr after the injection of reserpine. In another experiment in which reserpine had been administered to two dogs over a period of 4 days (0.5 mg/kg body weight on day 1 and 0.25 mg/kg body weight on days 3 and 4), it was found that on the fifth day the concentration of 5-OR indolyl compounds in the caudate nuclei (Table 3) had fallen

TABLE 3

EFFECT OF CHRONIC ADMINISTRATION OF RESERPINE ON THE CONCENTRATION OF TOTAL 5-OR INDOLYL COMPOUNDS IN THE CEREBROSPINAL FLUID AND THE CAUDATE NUCLEUS

Concentrations expressed as ng 5-hydroxyindol-3-ylacetic acid/ml. cerebrospinal fluid or ng 5-hydroxyindol-3-ylacetic acid/g tissue

Reserpine administered over 4 days	Concentration of total 5-OR indolyl compounds on 5th day after initial reserpine administration				
	Cerebrospinal fluid samples				Caudate nucleus
	1	2	3	4	
Dog 1	46	84	105	—	282
Dog 2	98	83	83	89	262
Control dog	39	63	51	80	1,020

to less than half of the control values shown in Tables 2 and 3. This experiment also gave information on the concentration of 5-OR indolyl compounds in the cerebrospinal fluid after chronic reserpine administration. Consecutive 2 ml. samples of cerebrospinal fluid were obtained from each animal under ether anaesthesia. The results are shown in Table 3, and show that after the chronic administration of reserpine the concentration of 5-OR indolyl compounds in the cerebrospinal fluid is at a normal or slightly elevated level despite the greatly reduced concentration in the tissues.

The absence of a fall in the concentration of 5-OR indolyl compounds in the caudate nucleus 2 hr after an injection of reserpine suggested further investigation.

It has been shown (Paasonen & Vogt, 1956) that there is a fall in the concentration of 5-hydroxytryptamine in the caudate nucleus of the dog 4.5 hr after an injection of reserpine in a dose of 0.5 mg/kg. Brodie (1958) demonstrated that in the rabbit a dose of 1 mg/kg of reserpine will deplete 75% of the 5-hydroxytryptamine in the brain in 30 min. Recently, Brodie, Finger, Orlans, Quinn & Sulser (1960) have reported that stress will inhibit the release of 5-hydroxytryptamine from the brain after the administration of reserpine. The possibility that the stress of anaesthesia and subsequent operations was preventing the release of 5-hydroxytryptamine in the foregoing experiments was therefore investigated.

Two dogs were trained for seven days to lie quietly while the preparations for an intravenous injection were carried out. On the eighth day they were injected intravenously with reserpine (2 mg/kg body weight). Within 30 min the animals were exhibiting effects of the reserpine such as tremor, vomiting and in one case circling movements. These symptoms disappeared after 15 min and the animals became sedated. 2.5 hr after the injection of reserpine the animals were anaesthetized and killed. The concentration of total 5-OR indolyl compounds in the caudate nuclei of these two animals was 740 ng/g and 755 ng/g. This result demonstrated that the concentration of 5-OR indolyl compounds in the caudate nucleus of the dog

TABLE 4

CONCENTRATION OF 5-OR INDOLYL COMPOUNDS IN THE CAUDATE NUCLEUS OF THE DOG

Concentrations expressed as ng 5-hydroxyindol-3-ylacetic acid/g tissue

	Total 5-OR indolyl compds	Acidic 5-OR indolyl compds	Basic 5-OR indolyl compds
Control dogs	610	184	485
	845	285	631
2 hr after reserpine (2 mg/kg intravenously)	650	478	172
	670	557	189

does not fall 2.5 hr after the injection of reserpine despite the fact that the reserpine had an obvious effect on the behaviour of the animal, and that any " stress " was avoided.

Sharman (1960) has shown that a substance behaving like 5-hydroxyindol-3-ylacetic acid can be extracted from dog brain tissue, and a second explanation was that 5-hydroxyindol-3-ylacetic acid, formed by the action of monoamine oxidase on the 5-hydroxytryptamine released by reserpine, was remaining in the brain tissue.

The following experiment was carried out on four litter-mate puppies. Two of the dogs were injected intravenously with reserpine (2 mg/kg body weight). After 2 hr the animals were anaesthetized, bled out and the caudate nuclei removed and analysed for total, acidic, and basic 5-OR indolyl compounds. The caudate nuclei from the two control dogs were analysed in a similar way. The results obtained are shown in Table 4. They show that an acidic indolyl compound, presumably 5-hydroxyindol-3-ylacetic acid, is increased by about as much as the 5-hydroxytryptamine is reduced after reserpine; the formation of this metabolite and its persistence in the tissue explain the absence of a reduction in the total 5-OR indolyl compounds 2 hr after reserpine.

Effect of imipramine on the concentration of 5-OR indolyl compounds in the cerebrospinal fluid

Marshall, Stirling, Tait & Todrick (1960) have demonstrated that imipramine produced a fall in the concentration of 5-hydroxytryptamine in the blood platelets in man. A reduction to about 50% of control levels was observed after this drug had been administered daily for 5 days. Costa (1960) has observed that imipramine will increase the 5-hydroxytryptamine content of several organs in the rat within 5 hr after the administration of the drug.

The effect of imipramine on the level of 5-OR indolyl compounds in the cerebrospinal fluid was examined. Preliminary experiments in which cerebrospinal fluid was collected by method 2 did not indicate acute changes in the level of 5-OR indolyl compounds in the cerebrospinal fluid after a single dose of imipramine.

The chronic effect of this drug on concentration of 5-OR indolyl compounds in cerebrospinal fluid was investigated using the third method of collection in which daily samples of cerebrospinal fluid were obtained from anaesthetized dogs. Control samples of cerebrospinal fluid were collected on four successive days from three dogs and on two successive days from one dog. After an interval of three days

TABLE 5

EFFECT OF IMIPRAMINE ON THE CONCENTRATION OF TOTAL 5-OR INDOLYL COMPOUNDS IN THE CEREBROSPINAL FLUID

Concentrations expressed as 5-hydroxyindol-3-ylacetic acid

Dog no.	Day of expt.	Conc. of total 5-OR indolyl compounds: In cerebrospinal fluid (ng/ml. cerebrospinal fluid)	Conc. of total 5-OR indolyl compounds: In caudate nucleus (ng/g tissue)
1	1	29	
Control dog	2	32	
	3	32	
	4	26	
	8	26	
	9	31	
	10	30	
	11	28	950
2	1	26	
Control dog	2	—	
	3	25	
	4	45	
	8	30	
	9	36	
	10	28	
	11	27	685
3	1	31	
Imipramine	2	33	
started on day 5.	6	49	
Blood observed in	7	89	
cerebrospinal fluid	8	118	
on day 6	9	56	1,190
4	1	26	
Imipramine started	2	32	
on day 7	3	26	
	4	25	
	8	30·5	
	9	36·0	
	10	35·0	1,065

two of the animals were injected intramuscularly with imipramine, twice daily, starting with 2 doses of 100 mg on the first day of injection, and increasing the doses on subsequent days by 50 mg per day to twice 250 mg on the fourth day of injection. One animal was then found to be suffering from gross oedema at the site of the injections, and was anaesthetized, bled out and the caudate nuclei dissected out from the brain. The other dog was therefore given the last dose by mouth. It was killed the following day. On each day during the experiment approximately 2 ml. of cerebrospinal fluid was obtained from each of these animals and also from the two control dogs. The results of this experiment are shown in Table 5. They show that there is only a small day-to-day variation in the concentration of 5-OR indolyl compounds in the cerebrospinal fluid of normal dogs. Bleeding into the cerebrospinal fluid occurred in one of the animals (no. 3) which was receiving imipramine. The cerebrospinal fluid on subsequent days was xanthochromic and the concentration of total 5-OR indolyl compound rose sharply probably because of the release of 5-hydroxytryptamine from blood platelets during clotting at the site of the bleeding. The observations on the remaining animal which had been treated with imipramine do not indicate any change in the concentration of total 5-OR indolyl compounds in the cerebrospinal fluid as a result of the administration of this drug. Imipramine did not produce a definite change in the concentration of total 5-OR indolyl compounds in the caudate nuclei in this experiment.

DISCUSSION

The experiments described here have shown that changes in the concentration of total 5-OR indolyl compounds in the cerebrospinal fluid can reflect changes in the metabolism of 5-hydroxytryptamine such as those occurring after the administration of reserpine. The changes in 5-hydroxytryptamine metabolism which were presumed to occur after imipramine were not reflected by any change in the cerebrospinal fluid level of 5-OR indolyl compounds.

The change in metabolism of 5-hydroxytryptamine after a single injection of a large dose of reserpine is very great and it is possible that smaller changes might not be obvious when the acute experimental methods described here are employed. The demonstration that the release of the metabolites of 5-hydroxytryptamine is a slow process led to the conclusion that the chronic experiment would be more useful in the detection of small changes in the metabolism of 5-hydroxytryptamine, provided that the change persisted for some time. Relatively small chronic changes should be detectable because the day-to-day variation in the concentration of total 5-OR indolyl compounds in the cerebrospinal fluid of normal animals is small. It is not possible to determine from these experiments the origin of the 5-OR indolyl compound or compounds appearing in the cerebrospinal fluid after reserpine treatment.

The observations on the total, acidic and basic 5-OR indolyl compounds in the caudate nucleus show that a better evaluation of the metabolism of these compounds in the tissues can be obtained by estimating all fractions. The main acidic 5-OR indolyl compound in brain is 5-hydroxyindol-3-ylacetic acid (Sharman, 1960) and the main basic compound is 5-hydroxytryptamine (Bogdanski *et al.*, 1956). There is the possibility that other acidic and basic compounds are present in the

brain. 5-Methoxyindol-3-ylacetic acid has been isolated from the pineal gland by Lerner, Case, Biemann, Heinzelman, Szmuszkovicz, Anthony & Krivis (1959), and other 5-OR indolyl derivatives such as *N*-acetyl-5-methoxytryptamine have also been isolated (Lerner, Case & Heinzelman, 1959). Because the methods used here would not distinguish all of these compounds it was found preferable to describe the substances by the general term of 5-OR indolyl compounds.

This work was carried out during the tenure by G. W. A. of a Medical Research Council Fellowship for training in clinical research.

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