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STIMULATION AND SENSITIZATION OF THE ISOLATED VENUS HEART BY CEREBROSPINAL FLUID

BY

M. B. BOWERS, JR.

From the Directorate of Medical Research, U.S. Army Chemical Research and Development Laboratories, Army Chemical Centre, Maryland

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5-Hydroxytryptamine was not detected in animal cerebrospinal fluid after amine oxidase inhibitors, reserpine, or both. Both human and animal cerebrospinal fluid can produce a stimulating effect on the clam heart which is not due to 5-hydroxytryptamine. Cerebrospinal fluid also has the property of increasing the sensitivity of the clam heart to 5-hydroxytryptamine.

Various indoles, notably 5-hydroxytryptamine, produce an increase in the contractile amplitude of the isolated heart of the clam *Venus mercenaria* (Welsh, 1957). This preparation has been used by several investigators to test for 5-hydroxytryptamine in cerebrospinal fluid, but the results have been conflicting (Turner & Mauss, 1959 ; Schain, 1960). In the present experiments cerebrospinal fluid, obtained from animals under various pharmacological conditions designed to increase or release brain 5-hydroxytryptamine, was assayed by the clam heart technique. In the course of these studies it became clear that cerebrospinal fluid alone could affect the clam heart directly and could alter the sensitivity of the heart to 5-hydroxytryptamine.

METHODS

In an attempt to increase the amount of 5-hydroxytryptamine in cerebrospinal fluid by pharmacological means, varying doses of 1-phenyl-2-hydrazinopropane hydrochloride (JB-516) and reserpine were injected by several routes into rabbits and dogs. At intervals cerebrospinal fluid samples were obtained by cisternal puncture under ether or nembutal anaesthesia. Human cerebrospinal fluid was obtained from samples collected during diagnostic pneumoencephalography. Only non-traumatic samples were used. Cerebrospinal fluid to be assayed for 5-hydroxytryptamine was tested on the day it was obtained or kept frozen until analysis. The technique for isolation and preparation of the clam heart was that of Welsh & Taub (1948). Contractions of the heart were recorded on an ink-writing kymograph. The sea-water bath was that described by Stewart (1952) except that calcium chloride was reduced by half to avoid precipitation of calcium salts at *p*H 7.2. Cerebrospinal fluid volumes of 0.2 to 1.0 ml. were added to the clam heart chamber and allowed to remain in contact with the heart 2 to 15 min before being washed out. Benzoquinonium chloride (Mytolon ; 2:5-bis (3′ diethylaminopropylamino)-benzoquinone bis benzyl chloride) 10^{-5} g/ml. was maintained in the bath except during the washing process. This drug is an effective antagonist of acetylcholine in the *Venus* heart (Luduena & Brown, 1952). In these experiments benzoquinonium was used to improve the regularity of the beat by preventing the depression of the heart caused by endogenous acetylcholine or by acetylcholine which might have been present in cerebrospinal fluid. Benzoquinonium has no effect on the response of the heart to 5-hydroxytryptamine. 5-Hydroxytryptamine creatinine sulphate stock solution was 2×10^{-4} g/ml. in 0.1 N HCl. Standards were prepared by diluting this

stock solution with sea-water. Doses and concentrations of drugs refer to the weight of the salt except reserpine, which is expressed as the free base.

RESULTS

Table 1 shows the pharmacological conditions used in an attempt to produce a detectable amount of 5-hydroxytryptamine in cerebrospinal fluid. Only the administration of 5-hydroxytryptophan (25 mg/kg intravenously) produced

TABLE 1

ABSENCE OF DETECTABLE 5-HYDROXYTRYPTAMINE ACTIVITY IN CEREBROSPINAL FLUID UNDER VARIOUS PHARMACOLOGICAL CONDITIONS

Cerebrospinal fluid sample time refers to hours after final drug administration. Sensitivity (mμg 5-hydroxytryptamine creatinine sulphate per ml. cerebrospinal fluid) refers to the lower limit of the assay for 5-hydroxytryptamine, using 1 ml. cerebrospinal fluid as maximum volume for the assay and considering a 5% increase in contractile amplitude the minimum which can be reliably detected. Sensitivity is shown before and after testing of cerebrospinal fluid samples for 5-hydroxytryptamine. Where two values are given, the assay was attempted on two *Venus* hearts. Number of animals for each condition shown in parentheses

Condition	Animals	Cerebrospinal fluid sample time	Sensitivity: Before cerebrospinal fluid	Sensitivity: After cerebrospinal fluid
A. Reserpine alone				
5 mg/kg intravenously	Rabbits (13)	1–5	20, 25	10, 12
B. JB-516 alone				
2 mg/kg/day subcutaneously for 6 days	Dogs (4)	4	30, 50	12, 20
30 mg/kg subcutaneously	Rabbits (6)	4	35	20
C. Reserpine after JB-516				
Reserpine 5 mg/kg intravenously 3 hr after JB-516 4 mg/kg subcutaneously	Rabbits (6)	3	25, 35	10, 20
Reserpine 2 mg/kg intravenously 24 hr after JB-516 2 mg/kg subcutaneously	Rabbits (6)	3	45	20

5-hydroxytryptamine-like activity in cerebrospinal fluid which was antagonized by 2-bromolysergic acid diethylamide; but 5-hydroxytryptophan itself stimulated the clam heart at concentrations that may have been present in cerebrospinal fluid after intravenous injection. Bogdanski, Weissbach & Udenfriend (1958), using fluorimetric methods, have found 5-hydroxytryptamine in cerebrospinal fluid after 5-hydroxytryptophan administration.

Although no 5-hydroxytryptamine activity was found under the conditions cited in Table 1, we frequently observed that cerebrospinal fluid produced a slow increase in contractile amplitude (Fig. 1a, b) which mimicked 5-hydroxytryptamine but was not blocked by 2-bromolysergic acid diethylamide, a specific 5-hydroxytryptamine antagonist on the clam heart. This effect was seen with 0.3 ml. or more cerebrospinal fluid. An 8 to 15% increase in contractile amplitude was usually produced by 0.5 ml. cerebrospinal fluid after 6 min. Repeated additions often gave a less pronounced response, suggesting tachyphylaxis. Concentration of the cerebrospinal fluid by freeze drying or air evaporation in the cold increased the activity of the stimulating property per unit volume of cerebrospinal fluid. The effect was only partially destroyed by heating cerebrospinal fluid in boiling water

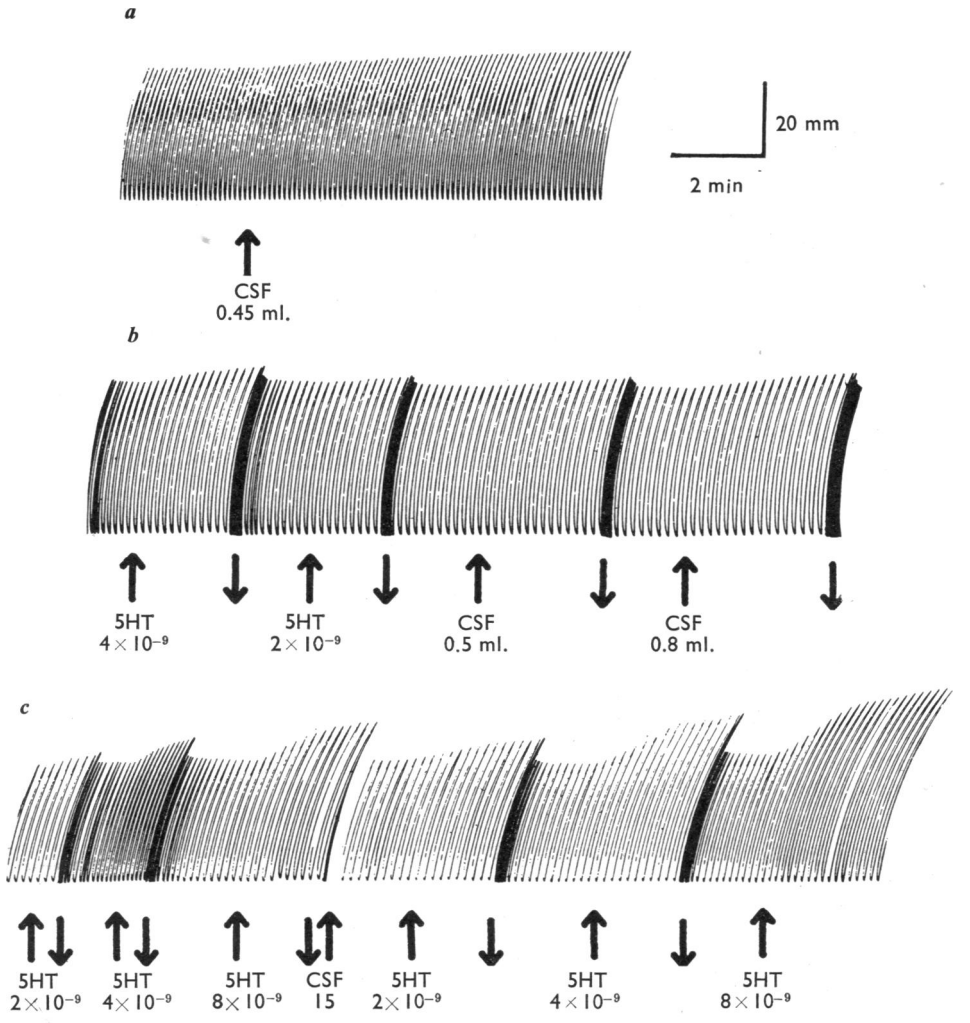

Fig. 1. The effects of human cerebrospinal fluid on the isolated *Venus* heart preparation. (*a*) The effect of 0.45 ml. cerebrospinal fluid (CSF) on contractile amplitude. Maximum effect obtained in approximately 6 min. (*b*) Difficulty distinguishing the stimulating effect of cerebrospinal fluid shown in (*a*) from true 5-hydroxytryptamine (5HT) during assay. 5-Hydroxytryptamine standards given as final chamber concentration. (*c*) Ability of cerebrospinal fluid to sensitize the *Venus* heart to 5-hydroxytryptamine. After the first three standard concentrations of 5-hydroxytryptamine, 0.8 ml. human cerebrospinal fluid was added to the chamber, allowed to remain 15 min, then removed by multiple washings. The 5-hydroxytryptamine standards were then repeated. Benzoquinonium chloride (Mytolon 10^{-6} g/ml.) was added to the chamber prior to each addition of 5-hydroxytryptamine or cerebrospinal fluid. Arrows pointing downward indicate washing. Chamber 5 ml., temperature 15° C.

for 10 min. I have found that increases in the potassium concentration in the bathing solution will increase the contractile amplitude of the *Venus* heart. However, several cerebrospinal fluid samples which showed stimulating activity on the heart contained only 3.5 to 4.0 m.equiv/l. of potassium ion. Addition of such samples to

the chamber would not increase the potassium concentration of the sea-water. Further, several cerebrospinal fluid samples containing large amounts of protein did not possess greater stimulating properties than samples containing normal amounts of protein.

Fig. 1c demonstrates the phenomenon of sensitization of the clam heart to 5-hydroxytryptamine produced by cerebrospinal fluid. When 0.1 to 0.8 ml. cerebrospinal fluid was added to the chamber, allowed to remain ten minutes, then removed by multiple washings, the clam heart became much more sensitive to 5-hydroxytryptamine standards. This increased sensitivity persisted for several hours. No such sensitizing effect could be demonstrated for acetylcholine. Both the stimulating effect and the sensitizing effect were most consistently seen with the freshest specimens of *V. mercenaria*. These effects seemed to be independent of one another, e.g., one effect could be present without the other in a given sample of cerebrospinal fluid. The stimulating and sensitizing properties were found both in human and animal (dog and rabbit) cerebrospinal fluid.

DISCUSSION

The effects of cerebrospinal fluid on the clam heart described here were first mentioned by Schain (1960), who was unable to confirm the report of Turner & Mauss (1959) concerning the presence of 5-hydroxytryptamine in human cerebrospinal fluid. Recently another report has cited values for 5-hydroxytryptamine in the cerebrospinal fluid of schizophrenic patients (Woolley & Campbell, 1962). These values are beyond the range of the usual sensitivity of the clam heart assay. It seems likely that the stimulating effect of raw cerebrospinal fluid on the clam heart has given rise to falsely positive assays for small quantities of 5-hydroxytryptamine in other reports. The sensitizing property is of interest in light of a previous report by Bhattacharya, Feldberg & Vogt (1957) which describes a substance in cerebrospinal fluid which sensitizes the frog rectus muscle to acetylcholine.

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