THE EFFECT OF AMPHETAMINE AND CHLORPROMAZINE ON THE ELECTROLYTE CONTENTS OF THE CEREBROSPINAL FLUID OF THE CAT

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ABSTRACT

Experiments were performed to investigate the effects of d-amphetamine and chlorpromazine on the electrolyte content of cerebrospinal fluid (CSF) withdrawn from the cisterna magna of the cat under chloralose anaesthesia. Electrical recordings from the cerebral cortex were also made. Amphetamine produced an increase in the potassium, sodium and to some extent chloride ions; while chlorpromazine had an opposite effect to that of d-amphetamine. Solutions containing these ions were also administered intracisternally to determine their effects on the electrical activity of the cerebral cortex. These solutions produced desychronization of the electrocorticogram which was similar to that caused by amphetamine. It is therefore suggested that the stimulant effect of amphetamine may be due in part to its ability to increase the cerebrospinal fluid content of these ions, while the effect of chlorpromazine may be due to the inhibition of the secretion of electrolytes into the cerebrospinal fluid.

INTRODUCTION

The formation, composition and absorption of CSF of cats and other species have been fully studied (Ames and co-workers, 1964; Bito and Davson, 1966) However, there have been no reports on the effects of psychotropic drugs on the electrolyte content of the CSF of the cat.

The role of sodium, potassium and chloride ions in peripheral neural function and brain electrical activity have been well documented (Carpenter and co-workers, 1968. Hodgin and Huxley, 1945). Potassium ions have been shown to be important in the release of neurotransmitter from synaptic junction. But whether an increase in these electrolytes in the cerebrospinal fluid affect the release of neurotransmitters and hence on brain function is not yet known.

Dahl (1968) showed that fatty acids inhibited rat brain sodium ions, and potassium – ATPase in vitro at concentrations similar to that causing narcosis in vivo. This could be interpreted as showing that the sleep-like state seen after fatty acid injection was a result of the inhibition of the sodium ions and potassium-ATPase of the brain, thus interfering with membrane repolarization.

Hughes and Brodie (1959) found that ouabain which blocks the Na+. ATPase system has been found to inhibit the uptake of biogenic amines. Potassium and sodium ions were found to be important in the uptake and storage of noradrenaline and serotonin (Bogdanski and Brodie, 1966). Eliel and co-workers (1950) studied post-operative mental disturbances and found that patients with decrease body potassium ions showed behavioural changes ranging from lethargy, apathy depression, nervousness, confussion to delirium.

In view of the fact that the brain, a relatively porous organ, is immersed in cerebrospinal fluid, it is reasonable to predict that the ionic content of the cerebrospinal fluid would affect neuronal electrolyte concentration. Since the neuron is the functional unit of the brain, drugs which affect the ionic concentration of the fluid in which it is bathed should affect brain function.

The purpose of this investigation, therefore, is to determine the effects of two psychotropic drugs, amphetamine and chlorpromazine, on the electrolyte content of the cerebrospinal fluid.

METHODS

All the experiments described in this investigation were performed on adult cats of both sexes. The cats weighed between 1.75 and 3.0 kg and they did not belong to any particular strain or breed as the animals were obtained locally from the market women. In all cases the ages of the animals were not known. A total of 25 cats were used for the investigation. All the experiments were performed on acute intact chloralosed -anaesthetized animal preparations.

PREPARATION OF ANIMAL AND OPERATIVE PROCEDURE

Anaesthesia was induced with 80 mg/kg chloralose. Tracheotomy was quickly performed, and artificial respiration was provided when necessary, using Harvard respiratory pump (Harvard Apparatus, Massachusettes, USA). The artificial ventilation was provided at 18 cycles per minute and 20cc/kg.

The animal was positioned in a stereotaxic apparatus (La Precision Cinamatographique, France). The skin overlying the top of the head was removed. The operation was carried out by separating and reflecting back the subcutaneous and temporalis muscles from the bone in the mid-line region of the skull. The periosteum was carefully removed with a bone scraper and any bleeding that occured was controlled by small quantities of bone surgical wax. Cortical electrodes, prepared according to the method previously described by Bradley and Elkes (1953), were placed on the primary auditory receiving areas of the cortex through burr holes made on one side of the skull with dental drill. Three other holes were made for electrodes to be inserted over the mid-ectosylvian, lateral and middle supra-sylvian gyri. The dura membrane was carefully pierced at each hole with a needle and

the six cortical electrodes screwed into place on the same side of the skull. The tip of each electrode just rested on the cortex. An earthing electrode was in the midline over the frontal sinus. The femoral vein was exposed and cannulated for the administration of drugs

GENERAL PROCEDURE

When all the cortical electrodes have been inserted, leads from the electrodes were soldered to the electrode leads of an eight-channel electroencephalograph machine (Schwarzer, Berlin, Germany). The animal was left in position for a period of thirty minutes before the experiment was started.

The normal electrocorticogram of the experimental animal under chloralose anaesthesia was recorded. The normal cerebrospinal fluid was then withdrawn. The second vertebra was located, the needle of a sterile 5 ml syringe was introduced at about the middle at 0.5 cm to 1.0 cm above this vertebra, in the place of a line running from this point through the external auditory to the nasion. The cisterna magna lies about 1.5 to 2.0 cm below the skin in cats. The needle was then gently moved upwards in a slanting position, once the archnoid membrane was punctured the animal jerked. A maximum of 2 ml of CSF was generally withdrawn at a time. Throughout this investigation, three hours interval was allowed between the withdrawal of the successive CSF. The CSF was withdrawn after the administration of each drug when there was a characteristic change in the electrocorticogram pattern, usually fifteen minutes after the administration of each drug.

At the end of each experiment the animal was sacrificed by the administration of an overdose of pentobarbitone intravenously.

Analytical procedure: Potassium and sodium ion determination.

The potassium and sodium ion concentrations were determined using a Clinical Flame Photometer, Corning – Eel Model 150.

A 1 in 200 dilution of the CSF was made with deionized water. The dilution factor was corrected automatically and the readings on the scale was expressed as Eq/litre.

Chloride Determination

The chloride ions were determined colorimetrically on the basis of the coloured ferric thiocynate formed when chloride ions react with an alcoholic solution of mercuric thiocynate in the presence of ferric alum. The colour produced was measured 470 nm (spectrophotometer Unican Sp 600 series 2). It became constant after 10 to 15 minutes and was stable for at least 2 h. The alcoholic mercuric thiocynate was prepared by dissolving 0.3 g mercuric thiocynate in 100 ml industrial methylated spirit. The alum solution was prepared by dissolving 6.0g ferric alum in 100ml of 6N nitric acid.

In the determination 10 ml of sample after 1 in 20 dilution was pipetted into a beaker previously rinsed with de-ionized water. Then 2.0 ml ferric alum solution and 1 ml alcoholic mercuric thiocynate solution was added. The absorbance was measured after 15 minutes at 470 nm. A calibration determination was carried out using concentration of 80 to 200 mEq/litre of sodium chloride. A graph of concentrations was ploted against absorbance. The concentrations of the cerebrospinal fluid were determined by extrapolating the absorbance to the curve and deducing the concentration.

The concentration of the two drugs used in the investigation, d-amphetamine sulphate and chlorpromazine hydrochloride were expressed in terms of their salts rather than the free base. Amphetamine sulphate was freshly prepared for each experiment while chlorpromazine hydrochloride was used in the solution supplied by the manufacturer.

RESULTS

It was observed throughout the experiments that tonic convulsions accompanied chloralose anaethesia. In all cases the animal entered into anaesthesia with the tongue protruding and the eyes widely opened. The anaestheuzed animal was still very sensitive to sensory stimulation, for example tapping of the table resulted in pronounced jerking response.

The electrocorticograms recorded in all the anaesthetized animals were similar.

AMPHETAMINE

Amphetamine sulphate was administered intravenously in doses of 1.0 mg/kg and 3 mg/kg, total dose. Desynchronisation of the electrocorticogram was used as an index of onset of action of the drug. The cerebrospinal fluid was withdrawn and the electrolyte determination is shown in table 1. Desynchronisation of the electrocorticogram occured about fifteen minutes after the administration of the drug.

About three minutes after the administration of 1 mg/kg amphetamine sulphate there was marked pupillary dilatation. The animal still responded to visual stimuli. Amphetamine produced about 40%, 150% and 1.4% increase in Na^+, K^+ and Cl^- content of the cerebrospinal fluid.

TABLE I

i. AMPHETAMINE

NUMBER OF	IONS	ELECTROLYTE CONTENTS OF THE CEREBROSPINAL FLUID IN mEq/litre				
		CONTROL	AMPHETAMINE 1mg/kg	AMPHETAMINE 3mg/kg	MEAN CHANGE IN ELECTROLYTE AMPHETAMINE 1mg/kg	AMPHETAMINE 3mg/kg
10	Na+	133.3+3.0	170.45+4	196.5+2.5	+35.15+1.2	+61.2+0.4
	K+	2.49+0.5	5.05+0.15	5.8+0.2	+2.56+0.3	+3.31+0.1
	Cl−	138.45+1.5	146.75+5.2	140.5+3.0	+8.30+0.1	+2.05+0.2

KEY:− + = INCREASE

CHLORPROMAZINE

Chlorpromazine hydrochloride was administered intravenously in doses of 2.5 mg/kg and 7.5 mg/kg, total dose. The cerebrospinal fluid was withdrawn and the electrolyte content was determined fifteen minutes after drug administration, at this time there was complete synchronization of the electrocorticogram. Chlorpromazine produced a decrease of about 20% sodium, 60% potassium and 5% chloride ions contents of the cerebrospinal fluid.

TABLE 2

ii. CHLORPROMAZINE

NUMBER OF EXPERIMENTS	IONS	ELECTROLYTE CONTENT OF THE CEREBROSPINAL FLUID IN mEq/litre				
		CONTROL	CHLORPROMAZINE 2.5mg/kg	CHLORPROMAZINE 7.5mg/kg	MEAN CHANGE IN ELECTROLYTE CHLORPROMAZINE 2.5mg/kg	CHLORPROMAZINE 7.5mg/kg
10	Na+	135.3+3.0	120.1+2.0	105+1.5	-15.2+0.1	-30+0.2
	K+	2.49+0.5	1.6+0.1	1.2+0.1	-0.89+0.05	-1.29+0.1
	Cl−	138.45+1.5	130+1.2	125+1.0	-8.45+0.2	-13.25+0.2

KEY:− - = DECREASE

ELECTROLYTES

The intracisternal perfussion with pottasium chloride solution, 10 to 15 mEq/litre produced marked dysynchronisation of the electrocorticogram. The dysynchronisation lasted about 30-45 minutes after the perfussion was stopped.

The intracisternal perfussion with 200 to 250 mEq /litre sodium chloride produced dysynchronisation of the electrocorticogram. The dysynchronisation lasted about 15 to 20 minutes after the perfussion was stopped.

DISCUSSION

The results of the present investigation show that amphetamine produces an increase in the electrolyte content of the cerebrospinal fluid. An increase in the extracellular concentration of Na+ and K+ have been shown to produce stimulation of neurons. It has been reported that intraventricular injection of 1% solution of potassium chloride will produce excitement and increase in locomotor activity (stern and co-workers 1922). Bogdanski (1968) reported that high concentrations of extracellular potassium ions, leads to influx of calcium ions and increase in the secretion of biogenic amines. Recent studies by Katzman and co-workers (1964) and Nakajima (1964) showed that intracisternal injection of potassium chloride produced agitation and excitation in rats, a phenomenon usually observed with amphatamine. These observations do favour the fact that amphetamine-induced behavioural stimulation coud be closely related to its ability to increase the electrolyte content of the cerebrospinal fluid.

It is known that intracisternal injection of potassium chloride in dogs produced varied cardiovascular effects such as hypertension, and cardiac arrhythmias as well as pulmonary ventifation. The amphetamine-induced increase in blood pressure and pulmonary ventilation may be as a consequence of the increase in the cerebrospinal fluid contents of K+ and Na+. The investigation of Downman (1943), Smolik (1943), Walker and coworkers (1945) Cooper and coworkers (1958) and Devos (1951) have confirmed these changes in the cardiovascular system by the intracisternal injection of these electrolytes. Calma and Wright (1947) reported that intracisternal injection of KCl produced pupillary dilatation, hypertension and respiratory irregularities. The pupillary dilation produced by amphetamine in choralosed cats may be

closely related to the increase in the CSF content of K+, and Na+. Glaser (1964) reported that intraventricular injection of hypertonic saline produced seizure discharges. The seizure discharges produced by amphetamine poisoning could be related to the increase in the CSF electrolyte content.

The studies with chlorpromazine showed that it decreases the electrolyte content of the CSF. One possible consequence of decrease in the extracellular concentration of these electrolyte is a decrease in the influx of Na+ which will prevent the generation of action potential. Inhibition of the generation of action potential would mean a depression of the electrical conductivity of the neurons involved. Siene the intracisternal injection of NaCl, and KCl produce E.E.G. arousal, changes in the concentrations of these electrolyte in the CSF could play an important role in the activity of these drugs.

REFERENCES

1. Ames, Sakanone and Endo (1964); Na+, K+, Ca++, Mg++ and Cl- concentrations in the choroid plexus fluid and the cisternal fluid, compared with plasma ultrafilterate, J. Neurophysiol. 27, 672-681.
2. Bito, L.Z and Davson, H. (1966), Local variations in cerebrospinal fluid composition and its relationship to the extra-cellular fluid of the cortex. Exp. Neurol. 14, 264-280.
3. Bogdanski, D.F., Blaszkowski, T.P. and Tissari, A.H. (1970); Mechanism of bigenic amine transport and storage iv Relationship between K+ and Na+ requirement for transport and storage of 5HT and Norepinephrine in synaptosomes. Biochim. Biophys. Acta, 211, 521-532.
4. Bogdanski, D.F. and Bordie, B.B. (1966); Role of Na+ and K+ in storage of norepinephrine by sympathomimetic nerve endings. Life Sci. 5, 1563-1569.
5. Bradley, P.B. and Elkes J. (1953), A technique for recording the electrical activity of the brain in the conscious animal. E.E.G. Clin. Neurophysiol 5, 451.
6. Calma, I. and Wright, S. (1947); Effects of Intrathecal injections of KCl and other solutions in cats. Excitatory actions of K+ on posterior nerve root fibres. J. Physiol. (Lond)., 106, 211-235.
7. Carpenter, A. and Alving, C. (1968); A contribution of an electrogenic sodium pump to membrane potential. J. Gen. Physiol. 52, 1-19.
8. Cooper, E.S., Hom, S. and Bellet, S. (1958); The cardiovascular effects following intracisternal potassium chloride infusion. Amer. J. Med. Sci. 235, 705-719.
9. Dahl, D.R. (1968); Short chain fatty acid inhibition of rat Na - K Adenosine Triphosphatase. J. Neurochem. 15, 815.
10. Devos, J (1951); Localisation of the potassium and calcium ions in the central nervous system. Arch. Int. Physiol. 59, 388-390.
11. Dowunman, C.B.B. and Mackenzie, C.C. (1943); Intracisternal injection of potassium phosphate Lancet, 2, 471-473.
12. Eliel, L.P., Pearson. O.H. and Rawson, R.W. (1950); Post-operative potassium deficit and metabolic alkalosis. New Eng. J. Med. 243, 471-478.
13. Hodgkin, A.L. and Huxley, A.F. (1945); Resting action Potential in nerve fibres. J. Physiol. (Lon) 104, 176-195.
14. Hughes, F.B. and Brodie, B.B. (1959); The mechanism of serotonin and catecholamine uptake by platelets. J. Pharm. Exp. Therap 127, 96.
15. Katzman, R., Weitzman, E.D., Graziani, L. and Escarivia, A. (1964); Ventriculocisternal perfussion of Rheus. Transport of Ca++ and K+ with correlated behavioural changes. Neurology 14, 247.
16. Nakajima, S. (1964); The effect of chemical injection into the reticular formation of rats. J. comp. Physiol. Psychol. 58, 10-15.
17. Smolik, E.A. (1943); Effect of intracisternal injection of potassium phosphate in hemorrhage hypotension and shock in the dog. Proc. Soc. Exp. Biol. Med. 53, 70.
18. Stern and Chvoles, G.J. (1933); Effect de l'injection intravenitriculaire des ions Ca++ et K+. C.R. Soc. Biol. (Paris) 122, 568-570.
19. Walker, S.M., Smolik, E.A. and Gilson, A.S. (1945); The effects of intracisternal injection of potassium phosphate on the rate and rhythm on the heart and the blood pressure and respiration of the dog. Amer. J. Physiol. 145, 225-238.

LEGENDS

Table 1: The effects of Amphetamine sulphate on the electrolyte contents of the cerebrospinal fluid.

Table 2: The effects of chlorpromazine on the electrolyte contents of the cerebrospinal fluid.