## CHAPTER VIII

### EFFECTS OF CAFFEINE ON BRAIN ACETYLCHOLINE (ESTIMATION OF RAT'S BRAIN ACETYLCHOLINE CONTENT FOLLOWING CAFFEINE TREATMENT BY FISH GUT BIO-ASSAY METHOD)

Although the principal aim of the thesis was to analyse the pharmacological actions of different chemical agents on the intestine of Ophicephalus punctatus, the preliminary studies with isolated perfused gut of the particular fish showed a remarkable sensitivity towards acetylcholine response. This encouraged us further to develop a sensitive method for the bio-assay of acetylcholine extracted from the biological tissues or fluids. Further experimentation enabled us to establish a suitable and simple procedure for the bio-assay of acetylcholine which appeared to be better in comparison with the available techniques, particularly that of frog's rectus abdominis preparation. This has been reported before. In order to assess the practicability of such a method an attempt has been made in this chapter, to estimate brain acetylcholine content of rat before and after the treatment of caffeine, by adapting the above procedure.

Caffeine was chosen because of its widely known cerebral cortical stimulant activity as reported by various workers (Berger,1937; Gibbs and Maltby, 1943). Caffeine has been reported to cause a significant increase in certical excitability along with the increase of oxygen consumption (Brooks et al,1949).

Maiti and Domino (1960 ; 1961) observed that caffeine in relatively small doses (on a basis of mg/kg), as contained in a cup of coffee, was very potent in prolonging the cortical after-discharges, both from intact and neuronally isolated cerebral cortex, which lasted for about 1 hour. It was not clear whether such effect of caffeine on the cerebral cortex

was related to its anti-cholinergic properties, particularly when caffeine was known to have anti-cholinesterase properties in muscles (Nachmanson, and Schueeman, 1945). It was also demonstrated by Holmstedt and Lundgren(1967) that increase in total amount of acetylcholine in rat's brain occured during increased excitation of cerebral cortex induced by tremorine and scopolamine which are potent anticholinergic drugs. Therefore, it appeared to be of great interest to study the level of the brain acetylcholine following caffeine treatment. Preliminary account of this work has already been published (Maiti and Seal, 1967).

Methods :

The experiments were carried out on 100 male albino rats (120-150gm) divided into several groups. Two from each group were used for recording the electrocortical activity through Grass EEG III machine by means of pin electrodes implanted in the skull over the frontal, parietal and occipital areas of both hemispheres, the animals were restrained in a rat-board and the head was fixed partly by head holder. Tracheostomy was performed for artificial respiration at the end of ether anaesthesia.After withdrawal of anaesthesia, while the normal EEG was being recorded, after-discharges were induced electrically by stimulating the parietal area through bipolar concentric electrodes with square wave pulses of 40 cps and 1 m.seconds duration for 3 seconds at 10minutes intervals. The threshold voltage for eliciting reproducible cortical after- discharges of similar duration recorded from the parietal and fromtal, and occasionally from occipital, areas varied between 2 to 4 volts as delivered from the $S_4$ Grass electronic stimulator. Submaximal stimuli were used in all animals to obtain cortical after-discharges of approximately similar durations from unanaesthetised flaxedil-treated (4mg/kg) artificially ventilated rats. Caffeine citrate solution in 0.1 ml volume, freshly prepared with normal saline

at pH 6.4, was injected through the tail vein in various doses (0.5 to 200 mg per kg body weight) at least 60 minutes after surgical manipulation and discontinuation of ether anaesthesia to all the groups except to that of control. After the electrical after-discharges were recorded at various intervals and at different time schedules after the administration of caffeine, the animals were killed by decapitation. Other animals were also decapitated at particular time schedule as followed in those used for EEG studies. After removing the brain, it was immediately weighed and placed in 2 volumes of ice-cold 10% TCA and minced finely with scissors. The total acetylcholine content was extracted from the cold homogenised sample by the method of Hebb (1963). To the diluted suspension of the minced tissue higher concentration of TCA (100 gm% solution) was added so that the final concentration stood at 10% TCA. This acid suspension was allowed to stand at room temperature for $1\frac{1}{2}$ hour with occasional stirring. It was then filtered and the precipitate was washed repeatedly with further addition of TCA and filtered. The combined filtrate was shaken 3 to 4 times with about 4 volumes of water saturated with ether until the extract reached pH 4.0 (just acid to congo red). The residual ether was blown off by aeration and the acetylcholine content of this final extract of cerebral cortex was estimated within 12 hours by bio-assay with the isolated eserine pre-treated fish intestine in a microbath as described previously. In one series of experiments, the homogenate was assayed for acetylcholine content using simultaneously both the fish intestine and the toads rectus preparation. Results ~~from~~ these two methods were in agreement in the normal rats and the rats pretreated with caffeine indicating that acetylcholine or a substance with acetylcholine like action has been measured.

Results :

1. Acetylcholine content of the cortical regions of cerebral cortex of normal rats.

A group of 12 rats were used for estimating the acetylcholine of normal brain without any treatment with caffeine. Instead of caffeine solution, 0.1 ml saline was injected through tail vein in them. The normal duration of electrical after-discharges with threshold stimuli at 4 volts varied from 10 to 15 seconds. The acetylcholine content of the cerebral cortex taken at different times as a control varied from 10 to 16 µ moles per gm tissue as illustrated in Figure 56. The acetylcholine content of symmetrical samples from the two hemispheres were more or less similar.

2. The effect of caffeine treatment on the brain acetylcholine content.

The experiments were conducted in two major groups. (i) In the first group, 48 rats were divided in four subgroups of 12 each to which caffeine was injected through the tail vein at 1 mg/kg, 10 mg/kg and 100 mg/kg. After recording the electrical after-discharges at different times the acetylcholine content of brain was measured at 10, 30, 60 and 90 minutes after caffeine administration. Figure 56 shows the time course of the increase in brain acetylcholine after the administration of caffeine.

Caffeine, starting from 1 mg to 100 mg/kg, caused a pronounced increase in the cortical after-discharges from the control value of 10 - 15 seconds to 40 seconds. Although the number of animals used was not very large, the significant increase in acetylcholine content of the brain tissue following caffeine treatment gave always a positive value. The maximal rise of acetylcholine occured in about 30 minutes and persisted

for more than 60 minutes, after which the level declined rapidly and reached below the normal value within 90 minutes. It was interesting to note that the increased acetylcholine content of cerebral cortex of rat was closely related with the appearance and disappearance of enhanced cortical afterdischarges. The rats treated with 100 mg of caffeine showed significant tremor but the maximum acetylcholine content was observed in 10 mg/kg dose.(Fig.56).

ii) In the second group, 48 rats were divided into 8 groups,6 rats in each. To each caffeine was given intravenously at increasing dosage, viz., 0.5 mg,1mg,5mg,10mg, 20 mg, 50 mg, 100 mg, and 200 mg/kg of the body weight, and the acetylcholine content of the brain was measured at 30 minutes after administration of the drug. Figure (55) illustrates the dose response curve showing the relative increase in acetylcholine with the dose of caffeine. The lowest dose that caused an increase in cortical afterdischarges was found to be 0.5mg/kg, but the minimal dose that produced an appreciable increase in acetylcholine content of cerebral cortex was 1 mg/kg. The highest dose which produced maximum increase in acetylcholine content of the brain was 10 mg/kg. But the maximum duration of cortical afterdischarges was noted in cases of rats following injection of 5 mg/kg.

Discussion :

The acetylcholine content of the brain can be influenced by various types of drugs; for example, many cholinesterase-inhibitors can increase the level of brain acetylcholine by 100%. In addition, the level of this hormone can be raised by barbiturate and other anaesthetics and lowered by convulsants. Atropine, scopolamine and certain other atropine like agents that exert a central action also cause a decrease in brain acetylcholine content (Giarman & Pepeu, 1962).

Caffeine in dosage of 1 mg/kg increases the content of acetylcholine of the cerebral cortex by as much as 20% by an action probably due to an inhibition of brain acetylcholinesterase. The time-course of the increased acetylcholine corresponds roughly to that of the pharmacological effects and this increase is counteracted by pretreatment of atropine. The evidence does not preclude the possiblity of the action of caffeine on the membrane of critical receptor sites of the cerebral cortical neurones apart from its direct anticholinesterase action.

Whatever might be the possible explanation for such increase in acetylcholine content of cerebral cortex following caffeine treatment, it is clear from the present study that there is a definite increase in acetylcholine content of brain following administration of caffeine at very low doses, although the tremorogenic effect of caffeine was observed with more than 100 mg of caffeine. However, the present observation suggests that the slight alteration of acetylcholine content, either in very minute amount or in a greater concentration, in the cerebral cortex of rat as caused by drug treatment could be comparatively assessed or analysed by using the isolated fish intestine bio-assay method.