

ACETYLCHOLINE LEVELS OF RAT BRAIN AND HEART IN STARVATION AND PROTEIN RESTRICTION

B. V. VENKATARAMAN, THANGAM JOSEPH, P. S. SHETTY* AND
P. M. STEPHEN**

Departments of Physiology and Pharmacology,
St. John's Medical College, Bangalore - 560 034*

and

*Departments of Pharmacology**
Christian Medical College Vellore - 632 002*

(Received on March 21, 1984)

Summary : Acetylcholine (ACh) levels and protein content in brain and heart were determined in normal, acutely starved, chronically semi-starved and chronically protein restricted groups of adult male rats. The only change observed in acute starvation and chronic semi-starvation was an increase in ACh level with a decrease in protein content in the heart, no change was observed in the brain. Protein restriction, however, produced a significant rise in ACh levels with a decrease in protein content of both brain and heart.

Key words : ACh protein content protein restriction starvation

INTRODUCTION

Acetylcholine (ACh) is a well documented neurotransmitter and dietary alterations can affect ACh levels (1). The availability of choline influences the synthesis of ACh in brain (2) and variation in ACh level of rat brain was observed during various periods of undernutrition (3). Landsberg and Young have attributed an important role to the sympathetic component of autonomic nervous system during dietary manipulation (4). This study was undertaken to examine changes in cholinergic transmitter levels if any, during dietary manipulations and whether responses of a central organ like the brain to such manipulation differed from that of a peripheral organ like the heart.

MATERIAL AND METHODS

Preliminary studies were undertaken to find out if there was any sex variation in ACh levels of brain and heart in normal rats. 10 male and 10 female adult six month old rats maintained on a diet of 4.2 cal/g were sacrificed by decapitation method (7) and ACh

extracted from brain and heart tissues by the method of MacIntosh and Perry (6). The samples were bioassayed on eviscerated and non-vagotomised rat's blood pressure (9). For the identification of unknown substance as ACh in the sample, eserine potentiation, atropine block, antihistamine and alkaline destruction tests were done. ACh content in whole tissue was expressed as nmol/tissue whereas ACh concentration was expressed as nmol/g. ACh levels in this context refers to both content and concentration.

The effect of dietary manipulation on ACh levels of brain and heart were studied in 60 fully grown adult male albino rats housed and maintained individually under uniform husbandary conditions. Control rats were fed *ad lib* on a mixed diet of 17% protein providing 4.2 cal/g. Acutely starved rats were deprived of food for 48 hr. Chronically semi-starved rats were given 50% of the amount consumed by the controls daily for 21 days. Chronically protein restricted rats were fed on a mixed diet of 4% protein providing 4.2 cal/g for 8 weeks which was isocaloric with its pair-fed control rats. All the rats had access to water *ad lib*. Acutely starved rats were in addition given electrolyte solution *ad lib* (4) to prevent the possible effect of salt restriction on ACh metabolism if any. At the end of the experimental period, test animals were sacrificed with their pair-fed controls for ACh estimation described earlier and protein content was determined by colorimetric method (5). Statistical analysis was done using student's 't' test.

RESULTS AND DISCUSSION

Perusal of Table I indicates that there was no significant change in ACh concentration of brain and heart between male and female rats; but total ACh of brain and heart in female rats was significantly lower ($P < 0.05$) than that of male rats. The higher total ACh content in male rats is probably due to higher weights of organs in the male rats ($P < 0.001$).

TABLE I : Acetylcholine levels in brain and heart of male and female rats.

Sex	Organ	Organ weight	Total ACh nmol/organ	Ach concentration nmol/g fresh tissue
Male (10)	Brain	1.5 \pm 0.01	23.7 \pm 0.60	15.5 \pm 0.30
	Heart	0.51 \pm 0.01	10.0 \pm 0.20	17.5 \pm 0.20
Female (10)	Brain	1.4 \pm 0.01**	21.6 \pm 0.50*	14.9 \pm 0.20
	Heart	0.53 \pm 0.01**	9.0 \pm 0.30*	17.0 \pm 0.30

* $P < 0.05$

** $P < 0.001$

Mean \pm S.E.

Figures in the parentheses represent number of animals.

TABLE II : Acetylcholine levels in brain and heart of normal, acutely starved, chronically semi-starved and chronically protein restricted rats.

Group	Organ	Protein content mg/g fresh tissue	Total ACh nmol/organ	ACh concentra- tion nmol/g fress tissue
Control (10)	Brain	96.0±0.60	23.5±0.60	15.7±0.50
	Heart	96.3±0.40	8.0±0.50	17.0±0.50
Acute starvation (10)	Brain	95.0±0.40	24.5±0.60	16.2±0.50
	Heart	95.3±0.60	8.8±0.50	17.7±0.50
Control (10)	Brain	96.±90.70	22.5±1.10	15.1±0.50
	Heart	96.9±0.30	9.5±0.50	17.1±0.50
Semi-starvation (10)	Brain	95.5±0.70	22.8±0.70	15.3±0.40
	Heart	94.9±0.70**	11.5±0.50*	20.0±0.60**
Control (10)	Brain	95.5±1.80	22.6±0.60	15.0±0.40
	Heart	95.8±1.00	9.0±0.60	16.8±0.70
Protein restriction (10)	Brain	89.0±1.40**	25.7±0.80**	17.0±0.40**
	Heart	88.7±0.90***	11.2±0.40**	20.8±0.40***

*P<0.02

**P<0.01

***P<0.001

Mean±Standard error

Numbers within parentheses indicate number of animals

Table II shows the ACh levels and protein content of brain and heart in different groups of rats. Acute starvation did not show any significant change in either ACh levels or protein content of the tissues studied. Chronic semi-starvation did not show any significant change in the brain ACh levels while heart showed a significant increase in both total ACh ($P<0.02$) and ACh concentration ($P<0.01$) and a significant decrease in protein content ($P<0.01$). Protein restriction, however, produced a significant increase in ACh levels of both brain ($P<0.01$) and heart ($P<0.001$). Protein content was also decreased significantly in both the organs ($P<0.01$; $P<0.001$ respectively). The percentage increase in ACh level of brain and heart (3–4% respectively) after acute starvation was not significant. After chronic starvation ACh level rises by 1% in the brain while in the heart the increase (17%) observed was significant. Hence ACh levels in peripheral organs such as heart seems to be more susceptible to change after semi-starvation. The lack of similar change in the brain is not surprising in view of metabolic priority enjoyed by the tissue. ACh level after protein restriction increased by 14% in the brain and 24% in the heart which were statistically significant. The increase in ACh levels may be due to an increased availability of acetylcoenzyme A from breakdown of fatty acids to replenish the energy supply to the animals. Decreased body activity and availability of choline during semi-starvation and protein restriction may also be responsible for the increase in ACh levels. It is worth noting that decrease in acetylcholinesterase activity of brain and heart reported earlier (8) bears good correlation to the changes in ACh levels reported

here. The brain, however, seems to have lost its stability after severe chronic protein deficiency though total caloric intake was kept constant.

ACKNOWLEDGEMENTS

Authors are grateful to the Indian Council of Medical Research, New Delhi and Research Society, St. John's Medical College, Bangalore, for financial aid; to Mrs. K. Sona Bai for technical assistance and Mrs. Saleena Joseph for secretarial assistance.

REFERENCES

1. Anderson, G.H. Diet, neurotransmitters and brain function. *Br. Med. Bull.*, **37** : 95-100, 1981.
2. Growdon, J.H. and R.J. Wurtman. Dietary influences on the synthesis of neurotransmitters in the brain. *Nutr. Rev.*, **37** : 129-136, 1979.
3. Kulkarni, A.B. and B.B. Gaitonde. Effects of early undernutrition and subsequent rehabilitation on acetylcholine levels in rat brain. *Experientia*, **38** : 377-378, 1982.
4. Landsberg, L. and J.B. Young. Fasting, feeding and regulation of the sympathetic nervous system. *N. Engl. J. Med.*, **298** : 1295-1300, 1978.
5. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193** : 267-275, 1951.
6. MacIntosh, F.C. and W.L.M. Perry. Biological estimation of acetylcholine. *Meth. Med. Res.*, **3** : 78-92, 1950.
7. Venkataraman, B.V., P.S. Shetty and Thangam Joseph. Variation in brain and heart acetylcholine content in rat; Cervical dislocation Vs guillotine technique. *Ind. J. Physiol. Pharmac.* **25** : 289-291, 1981.
8. Venkataraman, B.V., Thangam Joseph, P.S. Shetty and P.M. Stephen. Cholinesterase activity in starvation. *Ind. J. Physiol. Pharmac.*, **26** : 137-140, 1982.
8. Venkata Reddy, Y., P. Brahmayya Sastry and G. Ramadas. The effect of excess calcium on the acetylcholine turnover from the minced and incubated rat's brain. *Ind. J. Physiol. Pharmac.*, **22** : 285-292, 1978.