

# VARIATIONS IN BRAIN AND HEART ACETYLCHOLINE CONTENT IN RAT : CERVICAL DISLOCATION VS GUILLOTINE TECHNIQUE

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

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

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**Summary :** The stress induced in animals due to handling prior to sacrifice has been shown to produce changes in total acetylcholine (Ach) content of the brain and heart of adult rats. Two methods of killing the animals were compared viz. cervical dislocation and decapitation by a guillotine technique; the former method producing more handling stress than the latter. Ach levels were significantly lower both in brain and heart when the animals were killed by cervical dislocation.

**Key words:** handling stress      Ach      guillotine technique      cervical dislocation

## INTRODUCTION

Removal of tissues from animals for estimation of various biological substances are usually done after killing the animal by different methods. The stress induced by handling depends on the method used. The purpose of the present study was to determine quantitatively the total Ach content in whole brain and heart of rats killed by cervical dislocation and by decapitation using a guillotine technique, thus providing an useful comparison of the differences in handling stress in the two methods.

## MATERIALS AND METHODS

Twelve fully grown, 6 month old albino rats of either sex bred in our laboratory were used for this study. They were divided into two groups. Both groups of animals were on standard mixed diet *ad libitum* with free access to water. One group of rats were killed by cervical dislocation. This was done by grasping the head against the table firmly and pulling the tail with a single jerk and cutting off the head immediately with a large pair of scissors. The second group of rats were killed by a guillotine technique. Here the animal was allowed to move around to adjust to the environment and after steady-



ing the animal gently the head was chopped off by a smart blow behind the neck with the help of a heavy sharp edged cleaver thus reducing handling stress to a minimum.

Brain and heart tissues were rapidly excised and Ach was extracted by the modified procedure of MacIntosh and Perry (5). The extract so prepared was stable for atleast 2-3 days if kept at 0°C. However, in this study the samples were assayed within a few minutes of extraction to reduce loss of Ach, if any, either during storage or due to changes in pH.

*Assay:* Rat blood pressure preparation was used to assay the Ach content in the brain and heart tissues (2,10). Mepyramine maleate 1 mg/kg was injected iv to eliminate the possibility of the presence of histamine. The preparation was also sensitised by injection of eserine 0.05-0.1 mg/kg in 5% NaH<sub>2</sub>PO<sub>4</sub>. At regular intervals of 2-3 minutes alternating doses of unknown and standard solutions were matched by the method described by Burn *et al.* (3). At the end of the assay, it was confirmed that the depressor effect of the extract was destroyed by alkalization and that it was antagonised by atropine.

## RESULTS

Perusal of Table I indicates that the Ach content in both brain and heart of rats sacrificed by cervical dislocation was significantly lower ( $P < 0.001$ ) than in rats sacrificed by the guillotine technique employed.

TABLE I: Ach content in rat brain and heart after cervical dislocation and decapitation by guillotine technique.

Group	Method of sacrifice	Ach in $\mu\text{g/g}$ (Mean $\pm$ S.E.)	
		Brain	Heart
I	Cervical dislocation (5)	2.098 $\pm$ 0.147	2.066 $\pm$ 0.122
II	Decapitation by guillotine technique (6)	*3.076 $\pm$ 0.057	*3.873 $\pm$ 0.123

Figures in brackets indicates the number of rats.

\* $P < 0.001$

## DISCUSSION

Ach levels in the brain has been shown to vary with physiological states such as sleep (7), changes in body weight, sex and diet (6). Different types of stress also modify brain Ach content.



A reduction in Ach level in brain and heart was observed during spinning stress, cold stress and hypothermia and electrically induced convulsion (6,9,1,7) whereas an increase was observed during foot electroshock stress and restraint stress (4, 8). We have shown that handling stress alters the Ach levels in both brain and heart. Ach levels were significantly higher in rats sacrificed by the guillotine technique involving minimum of handling. It was evident that handling during cervical dislocation produced more stress as indicated by protrusion of tongue, bulging of eye balls, difficulty in respiration and convulsions.

The present study therefore indicates that the guillotine technique involving minimum of handling stress is a better method of sacrificing animals for estimation of Ach content in tissues.

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