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Changes in brain norepinephrine after decapitation

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ALTERATION in brain norepinephrine concentrations has been investigated in a wide variety of experiments. Invariably the tissue dissected out of a sacrificed animal is processed for norepinephrine determination after a definite postmortem period. If norepinephrine is not stable in "dead" brain, and the elapsed periods vary from experiment to experiment, an error is likely to appear in the results.

A cursory search in the literature revealed lack of information on this subject. It was therefore undertaken to determine the stability of endogenous norepinephrine in brain after decapitation.

Adult male Sprague-Dawley rats (Holtzman, Madison, Wis.) and adult male HA/IRC albino mice (Schmidt, Madison, Wis.) were decapitated and their brains dissected and weighed. One group of animals was sacrificed by instant freezing in a mixture of dry-ice and acetone (−78°). The brains were removed when frozen and homogenized immediately.

The control brains were homogenized immediately with trichloroacetic acid; the experimental brains were stored in uncovered glass beakers at room temperature (25 ± 1°) for predetermined periods before homogenization. A group of rat brains was stored in a closed chamber containing a water dish to provide a humid environment to prevent the loss of tissue water.

Brain norepinephrine was determined by the fluorometric procedure of Crout *et al.*[1] as modified by Harvey.[2] Each rat brain was assayed separately; three mouse brains were pooled. The control and experimental brains were always assayed simultaneously.

TABLE 1. EFFECT OF DEATH ON THE NOREPINEPHRINE CONTENT OF MOUSE BRAIN*

Time after decapitation	Norepinephrine			P
	Control	Experimental	Change (%)	
5 min	0·31 ± 0·009 (18)	0·28 ± 0·009 (18)	−10	<0·05
15 min	0·33 ± 0·011 (15)	0·26 ± 0·011 (18)	−21	<0·01
30 min	0·28 + 0·01 (18)	0·20 ± 0·016 (15)	−30	<0·01
1 hr	0·25 ± 0·009 (9)	0·14 ± 0·006 (9)	−44	<0·01
4 hr	0·29 ± 0·005 (15)	0·09 ± 0·007 (21)	−69	<0·01

* Amine concentrations are expressed in micrograms per gram of freshly dissected brain, mean ± S.E. The number of animals used is shown in parentheses.

It is seen from Table 1 that the norepinephrine content of the mouse brain decreased markedly after death. The norepinephrine contents of the brains homogenized at various time intervals after decapitation were significantly lower than those of the control brains which were homogenized in trichloroacetic acid immediately after decapitation. Qualitatively similar results were obtained when rats were used as experimental animals (Table 2). Data given in Table 3 show a similar fate of the brain norepinephrine when the loss of tissue water was prevented by storage in a humidity chamber. The loss of norepinephrine did not occur in brains frozen instantaneously and homogenized without thawing.

TABLE 2. EFFECT OF DEATH ON THE NOREPINEPHRINE CONTENT OF RAT BRAIN*

Time after decapitation	Loss of weight (%)	Norepinephrine	Change (%)	P
0	0	0·40 ± 0·013 (6)	0	
30 min	−5	0·34 ± 0·004 (3)	−15	<0·02
1 hr	−7	0·29 ± 0·019 (6)	−28	<0·01
4 hr	−21	0·19 ± 0·004 (3)	−53	<0·01

* See footnote to Table 1.

TABLE 3. EFFECT OF DEATH ON THE BRAIN NOREPINEPHRINE UNDER DIFFERENT STORAGE CONDITIONS

Animals	Experimental condition		Loss of weight (%)	Norepinephrine*	Change (%)	P
Rats	Normal†	Control	0	0·40 ± 0·013 (6)	0	
		Experimental	−21	0·19 ± 0·004 (3)	−53	<0·01
	Humidity chamber‡	Control	0	0·38 ± 0·003 (3)	0	
		Experimental	− 2	0·11 ± 0·009 (3)	−71	<0·01
	Instant freezing§	Control		0·40 ± 0·029 (4)	0	
		Experimental		0·41 ± 0·030 (4)	+3	N.S.
Mice	Normal†	Control		0·29 ± 0·005 (15)	0	
		Experimental		0·09 ± 0·007 (21)	−69	<0·01
	Instant freezing§	Control		0·33 ± 0·013 (9)		
		Experimental		0·32 ± 0·018 (9)	−3	N.S.

* See footnote to Table 1.
† Under normal laboratory conditions, 4 hr.
‡ In humidity chamber, 4 hr.
§ Frozen 4 hr before homogenization and homogenized without prior thawing.
N.S. = Not significant.

From exploratory experiments in guinea pigs (data not reported) Sano *et al.*[3] concluded that norepinephrine content of the brain does not drop until 24 hr after death. Our results with mice and rats are at variance with the conclusions of these workers. Our data agreed qualitatively with those of Joyce,[4] who showed depression of serotonin concentration in the rat brain after death. However, the rate of norepinephrine loss seems to be considerably greater than that of serotonin.

The loss of brain norepinephrine after death of the animal was not due to dehydration, because the significant loss occurred after death even at the times when the weight loss was negligible. Moreover when the loss of water was prevented by saturating the storage space with water vapor, the loss of norepinephrine could not be reversed.

Studies on stability of brain enzymes reponsible for biogenesis or metabolism of norepinephrine have not been done in "dead" tissues. That these enzymes are responsible for norepinephrine loss was not determined directly in this experiment. However, instant freezing, which generally depresses the enzyme activity, protected the brain norepinephrine. The group of mice and rats which were sacrificed by rapid freezing, stored in cold (−26°), and the brains analyzed without complete thawing showed no decrease in the brain norepinephrine.

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