results show that N-methylaspartic acid is one of the most active substances which exists naturally in the brain. It is suggested that N-methylaspartic acid may be an excitatory neurochemical transmitter and that CH_3-group may be important for excitation and depression of brain function.

Details of this work will be published elsewhere in Japanese.

Acknowledgements—Sincere gratitude is expressed to Professors Imaizumi and Hano of Osaka University for encouragement during this work.

Department of Pharmacology
Faculty of Pharmacy, Kinki University
Fuse, Osaka, Japan

T. KITA
H. KAMIYA
C. KIYOTA

REFERENCES

1. T. KITA, H. KAMIYA and C. KIYOTA, J.J.P.C. In press. (In Japanese.)
2. E. G. MCGEER, P. L. MCGEER and H. MCLENNAN, *J. Neurochem.* **158**, 296 (1961).
3. D. R. CURTIS and J. C. WATKINS, *Nature, Lond.* **191**, 1010 (1961).
4. T. J. HALEY and W. G. MCCORMICK, *Brit. J. Pharmacol.* **12**, 12 (1957).

Uptake of noradrenaline by subcellular particles in homogenates of rat brain*

(*Received* 10 *September* 1962; *accepted* 29 *October* 1962)

THE subcellular localization of catecholamines in particulate fractions prepared from portions of the central nervous system,[1, 2] the adrenal gland,[3–5] and postganglionic sympathetic nerve fibers (splenic nerves)[6–8] has suggested an integral role for the particulate entities in the uptake, storage, and release of these biogenic amines in neural tissue. The object of the present investigation was to determine whether subcellular particulate fractions obtained from the brain of the rat could concentrate and bind exogenous noradrenaline.

Subcellular fractions were prepared at 4° from freshly excised whole brains of decapitated male rats (200 g, Sprague-Dawley). Homogenization was performed for 65 sec in a glass homogenizer (Teflon pestle) containing an aqueous medium of sucrose (0·27 M) and edathamil (Versene, 0·1%); the final ratio of the sucrose medium to brain tissue was 9 to 1. All centrifugations were carried out at 4°. Nuclei and cell debris were removed by centrifugation at 900 × *g* for 10 min. The cloudy supernatant fraction (25-ml aliquot) was decanted and incubated with the priming amine for 30 min at 37°. The incubation mixture was then centrifuged at 100,000 × *g* for 30 min. The high-speed supernatant fraction was decanted and the sediment drained by inversion of the tube, the walls of the tube were wiped free from remaining supernatant fluid, and protein was removed from the sediment by the addition of 0·4 N perchloric acid (6 ml). The extract was passed through a cationic exchange column (Dowex 50-X8),[9] acetate-buffered at pH 6·0, and the catecholamine eluted with 1 N HCl. The noradrenaline content was estimated fluorometrically by a modification of the method of von Euler and Lishajko[10] in which ethylenediamine is added to the ascorbate-sodium hydroxide solution to stabilize the fluorophore.

The noradrenaline content of the final particulate fraction was 61·7 ± 9 (SEM) mμg/g whole brain (Fig. 1, A-1). Incubation of unprimed fractions at 37° for 30 min caused a marked decrease in the level of particulate-bound noradrenaline, whereas the loss occurring after incubation at 4° was minimal (Fig. 1, A-2, A3). The rate of release of endogenous catecholamine from splenic nerve[11] and adrenal medullary[4] granules is similarly temperature-dependent and accelerated at 37°.

The uptake of exogenous catecholamine by subcellular fractions was determined by adding noradrenaline (40 mμg/ml) to the incubation medium at zero time and estimating the noradrenaline

* This investigation was supported by a grant from the National Institutes of Mental Health (MH 03363-04).

content of the high-speed sediment after a 30-min incubation period. After such incubation, maximal uptake was observed and ranged from 3- to 4-fold above control levels (Fig. 1B). This increase may be attributed to a firm binding of the amine, since resuspension and further centrifugation of the high-speed sediment in 0·27 M sucrose caused no appreciable loss of particulate-bound catecholamine (5%). With particulate fractions containing low levels of endogenous catecholamines (induced by a

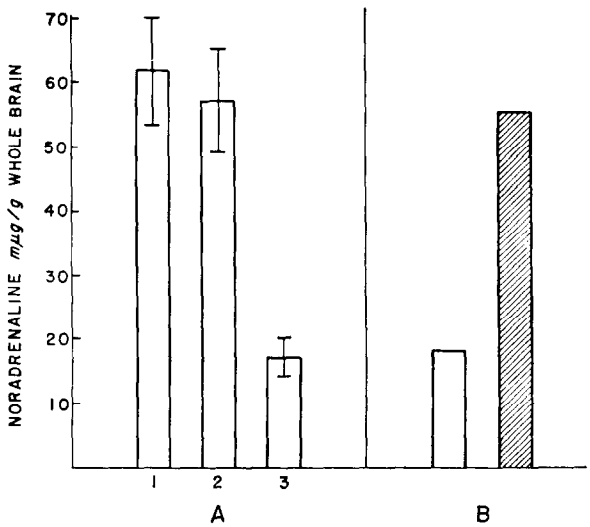

FIG. 1. A: Effect of incubation temperature on particulate-bound noradrenaline in the absence of exogenous priming amine: (1) zero time at 4°; (2) 60-min incubation at 4°; (3) 60-min incubation at 37°. Data taken from 12 experiments. B: Particulate-bound noradrenaline before (clear) and after (hatched) incubation of subcellular fraction with exogenous noradrenaline (40 mμg/ml). Incubation time, 30 min; temperature, 37°. Data taken from two experiments.

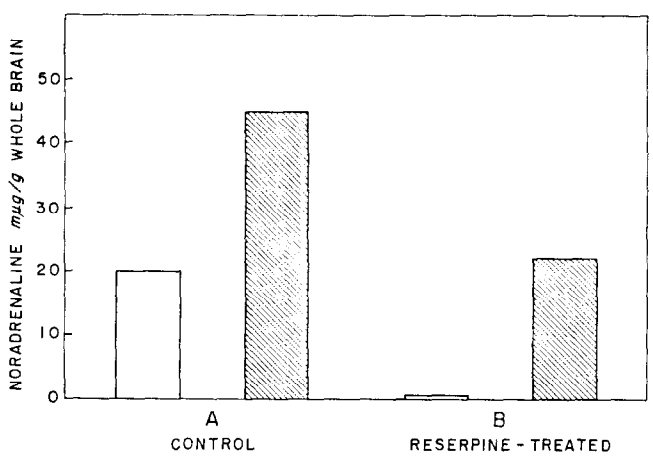

FIG. 2. Effect of reserpine (5 mg/kg intraperitoneally 24 hr prior to decapitation) on particulate-bound noradrenaline before (clear) and after (hatched) incubation of subcellular fractions with exogenous noradrenaline (40 mμg/ml). Incubation time, 30 min; temperature, 37°. Experiments involving control (A) and reserpine-treated (B) animals were performed simultaneously. Data represent mean of two experiments.

30-min incubation at 37°), uptake of exogenous catecholamine was similarly obtained. This uptake by preincubated subcellular fractions probably reflects the net difference between the rates of release and rates of concentration; interdependent but separate mechanisms may be found to control each of these processes.

The administration of reserpine to the rat (5 mg/kg i.p.) 24 hr prior to the experimental period clearly diminished the uptake-capacity of subcellular fractions prepared from these animals, but did not block it completely (Fig. 2).

These data demonstrate that subcellular fractions of rat brain possess a mechanism for concentrating exogenous noradrenaline similar to that shown for other neural structures.

Departments of Pharmacology and Psychiatry,
Yale University School of Medicine,
New Haven, Conn., U.S.A.

BERNARD L. MIRKIN*
NICHOLAS J. GIARMAN
DANIEL X. FREEDMAN

* Postdoctoral Fellow of the Laboratory of Psychopharmacology.

REFERENCES

1. A. BERTLER, N. A. HILLARP and E. ROSENGREN, *Acta physiol. scand.* **50**, 113 (1960).
2. H. WEIL-MALHERBE and A. D. BONE, *J. Neurochem.* **4**, 251 (1959).
3. H. BLASCHKO and A. D. WELCH, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **219**, 17 (1953).
4. N. A. HILLARP and B. NILSON, *Acta physiol. scand.* **31**, Supp. 113 (1954).
5. N. A. HILLARP, *Acta physiol. scand.* **47**, 271 (1958).
6. U. S. VON EULER and N. A. HILLARP, *Nature, Lond.* **177**, 44 (1956).
7. H. SCHUMANN, *Naunyn Schmiedebergs Arch. exp. Path. Pharmak.* **234**, 17 (1958).
8. U. S. VON EULER, *Acta physiol. scand.* **43**, 155 (1958).
9. A. BERTLER, A. CARLSSON and E. ROSENGREN, *Acta physiol. scand.* **44**, 273 (1958).
10. U. S. VON EULER and F. LISHAJKO, *Acta physiol. scand.* **51**, 348 (1961).
11. U. S. VON EULER and F. LISHAJKO, *Acta physiol. scand.* **51**, 193 (1961).

The effect of ricinoleic acid and phenolphthalein on creatine phosphate levels in the rat intestine *in vitro*

(*Received* 16 *October* 1962; *accepted* 4 *December* 1962)

MANY recent reports concerning movement of the intestine and intestinal smooth muscle have demonstrated the close relationship between the motility and the energy metabolism.[1-5] Some authors observed[6] that a high concentration of ricinoleic acid and phenolphthalein (10^{-2} to 10^{-3} M) inhibited respiration of intestinal slices of rats, whereas a lower concentration (10^{-6} to 10^{-7} M) stimulated the respiration slightly but significantly. It was probable that the respiratory stimulation by these drugs was caused by an interference with the formation of energy-rich phosphate bonds. The present paper therefore deals with the study of the action of these drugs on creatine phosphate levels in the rat intestine.

After adult albino rats, weighing 150 to 200 g, were sacrificed by decapitation, the intestinal tract was removed, opened lengthwise, and washed with saline. Both small and large intestine were used in this experiment; 300 to 700 mg of slices were incubated in 2 ml of Krebs-Ringer medium at pH 7·4 in which the concentration of Ca^{2+} was 0·001 M. At the end of the incubation period, the pieces of tissue were homogenized with two volumes of 10% trichloroacetic acid at −10° and then centrifuged at 0°. Free and total creatine were determined on aliquots of the supernatant by the colorimetric method of Eggleton *et al.*,[7] as used by Ennor and Rosenberg.[8] The difference between the two was taken as the measure of creatine phosphate. It was confirmed that the color formation was not affected by the concentration of the drugs used.