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Relative Lack of Pharmacologic Action of 3-Methoxy Analogue of Norepinephrine.* (23945)

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Investigations within the past year have indicated that a major pathway for metabolism of norepinephrine in the body is by a combination of methylation of the 3-hydroxy position and oxidative deamination. Armstrong, McMillan and Shaw(1) reported that 3-methoxy-4-hydroxy mandelic acid (VMA) is a major urinary metabolite of norepinephrine and epinephrine in man. Subsequently, Axelrod(2) showed that the 3-hydroxy positions of both of these amines can be methylated by animal tissues *in vitro* and *in vivo* to yield the corresponding 3-methoxy analogues. Axelrod *et al.*(3) have presented evidence that methylation *per se* and methylation followed by oxidative deamination are the major means of metabolizing norepinephrine administered to rats. It is still not certain, however, to what extent endogenous norepinephrine is metabolized in the same manner.

Recently, m-O-methylnorepinephrine (normetanephrine), presumably arising near the site of endogenous formation of norepinephrine, was found in adrenal gland and spleen (personal communication), in the brain of rats after administration of iproniazid(4), and in norepinephrine-producing tumors (pheochromocytomas) of man(5). It seemed important to determine whether this new amine would produce central or peripheral effects on administration to lower animals and to human subjects. The results to be reported suggest that O-methylation of norepinephrine *in vivo* results in its inactivation.

Methods. Studies of the neuropharmacological effects of normetanephrine (NMN) were carried out in cats. Clinical studies were conducted in 2 patients after observations in the dog suggested only weak pressor activity.

A. *Pharmacological studies in animals.* Studies of the effects of NMN on electrical activity of the brain were carried out in anesthetized cats, and in unanesthetized cats with permanently implanted electrodes. The anesthetized preparations (pentobarbital, 30 mg/kg, intraperitoneally) were employed in studies of the effects of intracarotid administration of NMN on the transcallosal response. In these preparations the transcallosal response was recorded from one suprasylvian gyrus following both maximal and submaximal electrical stimulation of a corresponding point on the contralateral hemisphere. NMN was administered *via* a small polyethylene catheter which had been placed in a thyroid branch of the common carotid artery and advanced until the orifice of the catheter was at the junction of the common carotid artery and the branch which had been cannulated. NMN was dissolved in isotonic NaCl solution; each injection was made in a volume of 1 ml. Chronic preparations with permanently implanted electrodes were employed to study the effects of the NMN on spontaneous cortical activity and on a variety of evoked cortical responses. The cortical response to a

* The normetanephrine was supplied as the hydrochloride through the generosity of Dr. Julius Axelrod and Dr. Bernard Witkop.

brief (10 μsec) light flash was recorded from the lateral gyrus. In other experiments the cortical response to electrical stimulation of lateral geniculate radiation fibers was studied. The effects of NMN on recruiting responses evoked by 6 to 12/sec stimulation of the nucleus reuniens(6) were also examined. The drugs employed in chronic preparations were administered intravenously. The technic employed in implantation of electrodes was that of Sheatz(7). Studies of the circulatory effects of NMN were carried out in a male, 15 kg mongrel dog under pentobarbital anesthesia. A polyethylene catheter was placed in a femoral vein for injections of drugs, and in a femoral artery for continuous recording of arterial pressure *via* a pressure-transducer-polyviso setup. Single injections of norepinephrine (5 μg) and NMN (1-4 mg) in 10 ml isotonic NaCl solution were given at 5 to 10-minute intervals.

B. *Clinical studies.* The human subjects (30-year-old white male weighing 70 kg and 46-year-old white female weighing 76 kg) were patients with uncomplicated labile hypertension. All observations were made with the subjects supine and in the fasting state. After a control period of one to 2 hours, alternate intravenous infusion of norepinephrine and placebo (5% dextrose in water) or NMN and placebo were given at 15 to 30-minute intervals with the subject being unaware of the nature of the injected material. Electrocardiograms (Lead II), radial pulse rate and arterial pressure (arm cuff-auscultatory technic) were recorded at frequent intervals during the control and experimental periods. A symptom questionnaire(8) was employed in the subject (30 yr. male) who received the largest dose (5 mg) of NMN in an attempt to detect any alteration of subjective state.

Results. NMN had no consistent effects on any of the forms of central electrical activity which were studied in cats. In the anesthetized preparations, intracarotid injections of up to 2 mg of NMN were without effect on the transcallosal response. The "spindling" which is characteristic of the spontaneous cortical activity during barbiturate anesthesia was unaffected by NMN. The studies carried out in unanesthetized cats

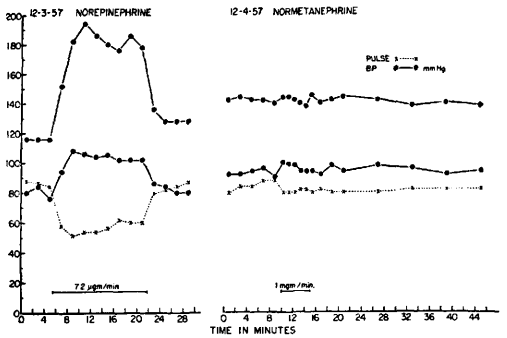

FIG. 1. Effects of norepinephrine and normetanephrine on blood pressure and pulse rate in a human subject. Drugs were administered intrav.

were also negative. NMN in doses up to 10 mg/kg intravenously was without effect on spontaneous cortical activity, recruiting responses, or the primary cortical responses to retinal photic stimulation or electrical stimulation of the lateral geniculate radiations. The gross behavior of the unanesthetized cats was not discernibly altered following intravenous administration of up to 10 mg/kg of NMN.

In the dog, with a maximum dosage of 4 mg NMN (0.25 mg/kg), a slight transient pressor response (+ 25/20 mm Hg) was observed. In contrast, 5 μg norepinephrine (0.3 μg/kg) produced a marked pressor response (+ 60/30 mm Hg). This single study suggested that NMN was 1/500th or less as active as norepinephrine on canine arterial pressure.

Varying doses of NMN were administered as 5-minute infusions in the 2 patients, beginning with 25 μg and to a maximum dose of 5 mg. No physiologic or psychologic reactions were observed. The marked cardiovascular reaction to norepinephrine (36 μg in 5 minutes) and the lack of any response to normetanephrine (5 mg in 5 minutes) is illustrated in Fig. 1.

Conclusions. Normetanephrine is inert as regards those aspects of central nervous system activity which have been studied in cats, and is a relatively weak pressor agent in the dog. An intravenous dose of 5 mg was inactive with regard to circulatory and psychological effects in man. Although a more detailed study of this compound should be done, the findings presented strongly suggest that O-

methylation at the 3 position is an effective means of inactivating norepinephrine. It will be of interest to investigate the enzymatic mechanisms involved in this unusual methylation process and to attempt to alter it *in vitro* and *in vivo*.

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A New Chemical Method for Measuring Cell Populations in Tissue Cultures.* (23946)

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Among more popular criteria for measuring cell populations in tissue cultures are cell counts, respiration rate, "hematocrit," isotope incorporation, nutrient uptake, desoxyribonucleic acid (DNA) content, and protein content (1-7,9-13). In this report we wish to present a new chemical method for measuring size of tissue cultures, based upon determination of "total purines and pyrimidines" (TPP). TPP is determined simply by extracting washed cultures with hot acid salt solution, and measuring the absorbance of the extract at 268 mμ. While TPP values correlate well with cell counts, mainly we have used DNA as a "standard of reference" for measuring populations. The case for DNA as a measure of cell numbers was summarized by Healy, Fisher, and Parker(6). To evaluate TPP as a measure of cell populations we studied young, old, starved and well-fed cultures, and determined the TPP, DNA and protein in each. As would be expected, TPP and protein both varied somewhat and similarly with respect to DNA. The data indicate that TPP is at least as good as protein for quantitation of cell populations. The virtues of the TPP method are simplicity, reproducibility, stability of absorbance, and a wide range of operation. Ribonucleic acid (RNA) determination is a logical extension of the TPP method, which we hope to employ. If the cells are first extracted with cold trichloracetic or perchloric acid, the TPP of the residue will consist of only RNA and DNA. RNA then will be equal to the difference between TPP and DNA, both being determined in the same alkaline extract as described below.

Methods. Cell cultures. HeLa S-3, was obtained from Tuskegee Institute. The McCoy strain of human cells was kindly supplied by Prof. C. M. Pomerat. Strain L, NCTC929-209-7 was obtained through the kindness of Dr. Wilton R. Earle, Nat. Cancer Inst. Stock cultures were fed and divided twice weekly. Human cells were cultured in a synthetic medium very similar to Eagle's, with human serum non-dialyzable fraction equivalent to 10% serum, supplemented with 1 g of Difco Bacto Tryptose/liter. Strain L was cultured in the same synthetic medium with 10% horse serum instead of human serum. Incubation was at 37°C. Replicate cultures were planted by Earle's apparatus (4). Strain L and McCoy were suspended by mechanical agitation; HeLa required trypsin treatment. Cultures were planted in 18 x 150 mm test tubes graduated for 5 ml volume, with a conically tapered bottom and an indentation 7 mm deep and 15 mm from mouth

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