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Some factors influencing the release of 5-hydroxyindol-3-ylacetic acid in the forebrain

A. PADJEN AND MIRJANA RANDIĆ

Biology Division, Institute "Rudjer Bošković", Zagreb, Yugoslavia

Summary

1. Electrical stimulation of the mid-brain raphé in anaesthetized adrenalectomized rats produced a significant decrease in the forebrain content of 5-hydroxytryptamine (5-HT) and an increase in the concentration of 5-hydroxyindol-3-ylacetic acid (5-HIAA).

2. Stimulation of peripheral sensory nerves did not influence either the forebrain content of 5-HIAA or the efflux of 5-HIAA from the cerebral cortex.

3. Probenecid (200 mg/kg) caused a twofold increase in 5-HIAA content of the rat's forebrain, while the efflux of 5-HIAA from the cerebral cortex remained unchanged.

4. Stimulation of the mid-brain raphé in animals pretreated with probenecid does not produce the rise in the forebrain levels of 5-HIAA seen in stimulated untreated controls and does not affect the efflux of 5-HIAA from the cerebral cortex.

5. In preliminary experiments, lysergic acid diethylamide (LSD 25) substantially reduced and/or prevented the increase in the release of 5-HIAA in the forebrain observed in untreated animals with raphé stimulation.

Introduction

Electrical stimulation of the mid-brain raphé, an area in which neuronal perikarya containing 5-hydroxytryptamine (5-HT) are almost exclusively located (Dahlström & Fuxe, 1964), produces a significant increase in the forebrain content of 5-hydroxyindol-3-ylacetic acid (5-HIAA) (Aghajanian, Rosecrans & Sheard, 1967; Sheard & Aghajanian, 1968; Eccleston, Padjen & Randić, 1969; Kostowski, Giacalone, Garattini & Valzelli, 1969). This increase in forebrain 5-HIAA is accompanied by an increase in the efflux of 5-HIAA from the cerebral cortex (Eccleston *et al.*, 1969). In accordance with the findings of Sheard & Aghajanian (1968) we have observed that the concentration of 5-HT did not change in the forebrain with stimulation (Eccleston *et al.*, 1969). These findings suggest that 5-HT in the brain is released by stimulation of a specific neural pathway projecting from the 5-HT-containing neurones in the mid-brain raphé to the forebrain and the cerebral cortex. The existence of such a pathway has been revealed by histochemical (Andén, Dahlström, Fuxe, Larsson, Olson & Ungerstedt, 1966) and combined anatomical and biochemical techniques (Heller, Harvey & Moore, 1962; Heller & Moore, 1965).

The present investigation was undertaken to determine whether the described changes in 5-HIAA on stimulation of n. raphé were specific for this region. In this paper we have shown that the increased release of 5-HIAA in the forebrain accompanied by a definite rise in the efflux of 5-HIAA from the cerebral cortex appears to be specific for stimulation of the mid-brain raphé region. This effect is present also in bilaterally adrenalectomized animals. However, there is no increase in the content of 5-HIAA in the forebrain and/or increase in efflux from the cerebral cortex on electrical stimulation of peripheral sensory nerves. The effect is also absent in probenecid-pretreated rats. This drug is known to block the transport of 5-HIAA out of rat brain *in vivo* (Neff, Tozer & Brodie, 1964, 1967; Werdinius, 1967; Eccleston *et al.*, 1969). In preliminary experiments lysergic acid diethylamide (LSD 25) reduced or blocked elevation of 5-HIAA in forebrain on stimulation of n. raphé.

Methods

Experiments were performed on adult male rats (200–250 g) lightly anaesthetized with urethane given intraperitoneally (1·0 g/kg of weight). Efflux of 5-HIAA from the cerebral cortex was studied by a technique similar to that described by Macintosh & Oborin (1953). Parts of the somatosensory, visual and auditory cortex were exposed bilaterally and after removal of the dura mater a light Perspex cylinder was placed on to the pial surface (0·3 cm^2) of each cerebral hemisphere. Both cylinders were filled with 0·25 ml of Krebs solution heated to 37° C. Leakage of fluid from the cylinders was prevented by using silicone grease. The Krebs solution was left in contact with the cortical tissue for periods of 30 min. It was necessary to pool four such samples to have enough 5-HIAA for reliable biochemical estimation. The resting and neurally evoked release of 5-HIAA was studied in separate animals. This procedure was adopted because in some preliminary experiments we found the 5-HIAA efflux increased steadily over the course of the experiment in the absence of stimulation. Samples were cooled to −20° C until the 5-HIAA was estimated spectrophotofluorimetrically (Ashcroft & Sharman, 1962). A bipolar steel stimulating electrode was placed stereotaxically into the caudal mid-brain raphé (using the rat brain atlas of König & Klippel, 1963) in both experimental (stimulated) and control (unstimulated) rats at A (ant.) 0·4 mm; L (lat.) 0·0 mm and H (horiz.) 2·6 mm. The correct placement of the electrodes was confirmed histologically in each animal. The stimuli were monophasic and/or biphasic square wave pulses (10 Hz, 2 ms in duration, 4–6 V) applied for 2 h from a Grass S8 stimulator. The peripheral sensory nerves were stimulated electrically through two stainless-steel electrodes inserted into the skin of a forepaw and the efficiency of stimulation was checked by monitoring the evoked potentials on a cathode-ray oscilloscope. Immediately after each experiment, the rats were decapitated and their brains rapidly dissected. The forebrain was removed from the brain-stem by a section passing between the rostral border of the superior colliculi and the caudal border of the hypothalamus. The contents of 5-HT and 5-HIAA in the forebrain were measured spectrophotofluorimetrically using a modification of the procedure described by Oates (1961) and Ashcroft & Sharman (1962).

Bilateral adrenalectomy was performed in rats by a standard lumbar approach under ether anaesthesia. The animals were maintained on 0·9% saline 5–12 days before the experiment.

Probenecid (Merck, Sharp & Dohme, N.J.) was administered intraperitoneally (200 mg/kg) 2 h before electrical stimulation of n. raphé. Lysergic acid diethylamide (LSD 25, Sandoz) was given intraperitoneally (0·5 mg/kg) 1 h before stimulation. The results were analysed statistically by using Student's *t* test.

Results

Effect of stimulation of n. raphé in adrenalectomized animals

We have shown previously that electrical stimulation of the mid-brain raphé consistently increased the forebrain content of 5-HIAA, while the concentration of 5-HT did not change with stimulation (Eccleston *et al.*, 1969). A similar increase of the forebrain content of 5-HIAA following mid-brain stimulation was also found in bilaterally adrenalectomized rats (Fig. 1). In contrast with non-adrenalectomized rats, however, in mid-brain stimulated adrenalectomized animals the forebrain content of 5-HT was significantly reduced by about 25%. The similarity of 5-HIAA results in controls and adrenalectomized animals excludes the possibility that non-specific " stress reaction " mechanisms involving the adrenal cortex are responsible for the rise of 5-HIAA.

We were unable to detect any significant change in the levels of 5-HT and 5-HIAA in the rat forebrain following bilateral adrenalectomy. Thus our results are in accordance with experimental data reported by Towne & Sherman (1960), Garattini, Lamesta, Mortari, Palma & Valzelli (1961), Resnick, Smith & Gray (1961) and Pfeifer, Vizi, Satory & Galambos (1963).

Effect of stimulation of peripheral sensory nerves

Electrical stimulation of peripheral sensory nerves did not influence either the forebrain content of 5-HIAA or the efflux of 5-HIAA from the cerebral cortex

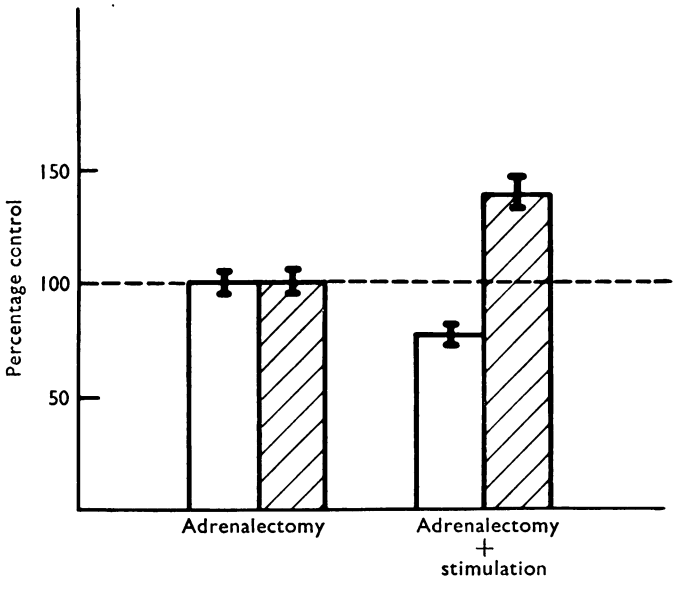

FIG. 1. Percentage changes in the levels of 5-HT (□) and 5-HIAA (▨) in the forebrain on electrical stimulation of mid-brain raphé in adrenalectomized rats. Values shown are the means (±S.E.) of five determinations.

(Fig. 2). The results of these experiments confirm and extend our previous findings (Randić & Padjen, 1968 ; Eccleston *et al.*, 1969 ; Eccleston, Randić, Roberts & Straughan, 1969a), suggesting a specific relationship between the electrical stimulation of the caudal mid-brain raphé and the release of 5-HT and/or 5-HIAA (the main metabolite of 5-HT) in the forebrain.

Effect of probenecid

When rats were treated with probenecid there was a twofold increase in 5-HIAA content of the rat's forebrain, as reported by Neff *et al.* (1967), while the efflux of 5-HIAA from the cerebral cortex into the cup fluid remained unchanged (Fig. 3). This latter finding confirms the suggestion (Neff *et al.*, 1964, 1967 ; Werdinius, 1967 ; Eccleston *et al.*, 1969) that 5-HIAA is probably transferred out of the rat brain by some active transport mechanism.

As shown in Fig. 4, electrical stimulation of the mid-brain raphé in animals pretreated with probenecid does not affect the efflux of 5-HIAA from the cerebral cortex and does not produce the rise in the forebrain levels of 5-HIAA which is seen in stimulated untreated controls (Aghajanian *et al.*, 1967 ; Eccleston *et al.*, 1969).

In four preliminary experiments, lysergic acid diethylamide substantially reduced or completely prevented the increase in the release of 5-HIAA in the forebrain observed in untreated controls with mid-brain raphé stimulation.

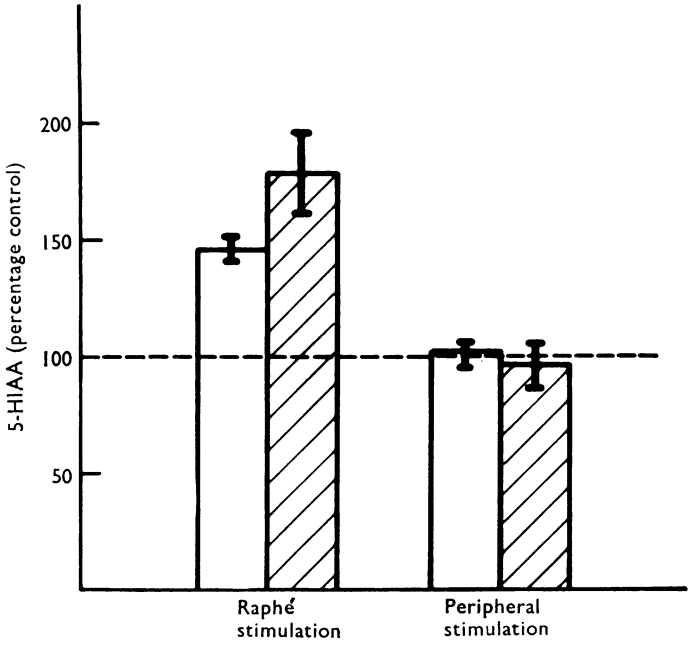

FIG. 2. Percentage changes in the levels of 5-HIAA in the forebrain (□) and the effluent from the cerebral cortex (cups) (▨) in rats on electrical stimulation of n. raphé and peripheral sensory nerves. Each histogram is the mean (±S.E.) of ten determinations.

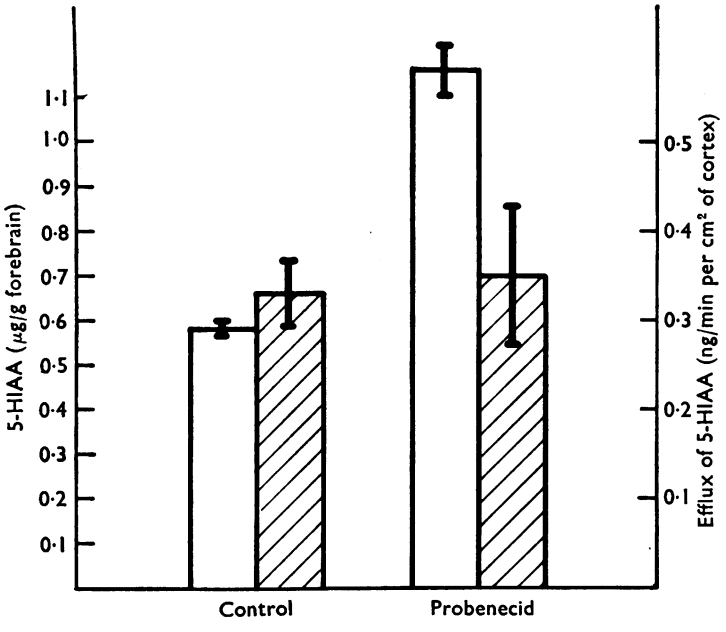

FIG. 3. Levels of 5-HIAA in the forebrain (□) and the effluent from the cerebral cortex (cups) (▨) in normal and probenecid-pretreated rats. Values shown are the means (±S.E.) of five determinations.

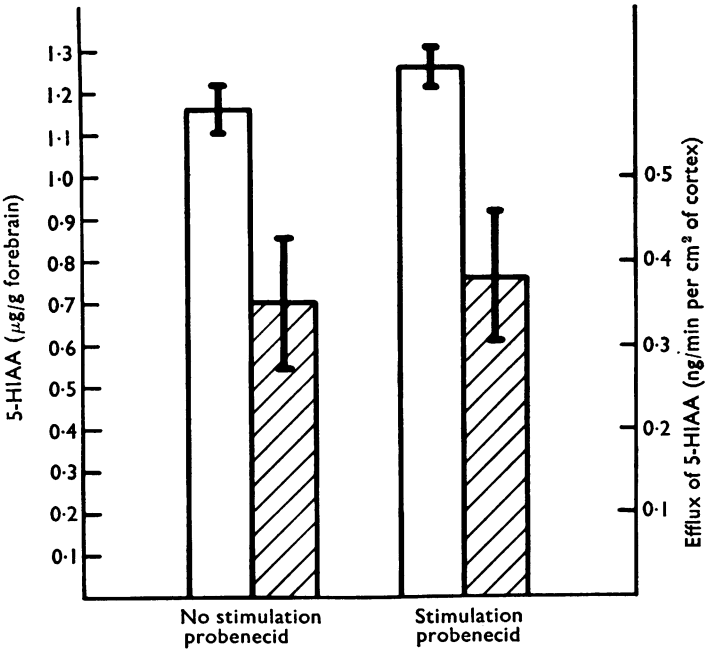

FIG. 4. Levels of 5-HIAA in the forebrain (□) and the effluent from the cerebral cortex (▨) on electrical stimulation of n. raphé in probenecid-treated rats. Each histogram is the mean (±S.E.) of five determinations.

Discussion

Using push-pull cannulae and cortical cup techniques Randić & Padjen (1968) and Eccleston *et al.* (1969a) were first to show in cats that there is a small but definite release of 5-HT in the lateral hypothalamus and the cerebral cortex. Electrical stimulation of the mid-brain raphé doubled or trebled the release of 5-HT in those structures of the brain. Eccleston *et al.* (1969) obtained a clear increase in the efflux of 5-HIAA from the cerebral cortex of rats with stimulation of the raphé region.

In this paper we have shown that electrical stimulation of the mid-brain raphé in adrenalectomized animals causes a significant reduction in the forebrain level of 5-HT and an increase in the level of 5-HIAA. The observed decrease in 5-HT content of the forebrain of adrenalectomized rats in our experiments might indicate that the biosynthesis of 5-HT does not keep pace with amine release on nervous stimulation. This finding differs from the results of Eccleston *et al.* (1969) in intact rats, where no change in the concentration of 5-HT of the forebrain with raphé stimulation was found. On the basis of the latter result we suggested (Eccleston *et al.*, 1969) that biosynthesis of 5-HT might be increased by central nervous stimulation as occurs with peripheral adrenergic nerves (von Euler, 1962 ; Roth, Stjärne & von Euler, 1966 ; Weiner & Alousi, 1966 ; Gordon, Reid, Sjoerdsma & Udenfriend, 1966 ; Sedvall & Kopin, 1967) and the spinal cord (Andén, Carlsson, Hillarp & Magnusson, 1964, 1965). Rosecrans & Sheard (1969) found that acute stress greatly increased 5-HT turnover in the rat brain tissue, providing that steady-state amine levels were below normal (in animals pretreated with *p*-chlorophenylalanine). They suggested that under conditions of stress the increased production of 5-HIAA most probably was due to a more rapid 5-HT synthesis and release rate and/or to the operation of a negative-feedback system in forebrain 5-HT-neurones.

Furthermore, we have analysed the specificity of the 5-HIAA changes described by Eccleston *et al.* (1969). We have found that electrical stimulation of peripheral sensory nerves does not alter either the forebrain content of 5-HT and/or 5-HIAA or the efflux of 5-HIAA from the cerebral cortex into the cup fluid. This suggests the existence of a specific relationship between stimulation of the caudal mid-brain raphé (n. raphé dorsalis, n. raphé medianus) and the release of 5-HT and/or 5-HIAA in the forebrain and 5-HIAA from the cerebral cortex (Padjen & Randić, 1969).

In a previous paper (Eccleston *et al.*, 1969) we showed that there is about a two-fold increase in 5-HIAA content of rat forebrain 2 h after pretreatment with probenecid (200 mg/kg), confirming the observations made by other workers (Neff *et al.*, 1964, 1967 ; Werdinius, 1967). We found, moreover, that the efflux of 5-HIAA from the cerebral cortex into the cup fluid remained unchanged in the presence of the increased levels of 5-HIAA in the forebrain of the probenecid-pretreated rats. The latter finding suggested that 5-HIAA was probably transferred from the brain into the cup-fluid by some active transport mechanism. The fact that probenecid did not abolish completely the efflux of 5-HIAA from the cerebral cortex suggests that only a part of 5-HIAA found in the effluent is actively transported. In contrast with the effect seen in untreated animals raphé stimulation, as reported in the **Results**, caused no rise in the forebrain levels of 5-HIAA from the cerebral cortex in probenecid pretreated rats. Sheard & Aghajanian (1968) also observed some

reduction in the increase of the release of 5-HIAA in rat's forebrain with raphé stimulation.

Probenecid reduces the renal excretion of 5-HIAA (Despopoulos & Weissbach, 1957), possibly by interfering competitively with the formation of an intermediate complex involved in the transport of organic acids through the renal tubular cell. There is an active transport system for the removal of 5-HIAA from the brains of rats, mice and cats (Neff *et al.*, 1964, 1967 ; Werdinius, 1967 ; Sharman, 1966 ; Reid, Volicer & Brodie, 1968). In cats (Bowers & Gerbode, 1968) and dogs (Ashcroft, Dow & Moir, 1968) 5-HIAA is actively removed from the cerebrospinal fluid and a low dose of probenecid allows systemically administered 5-HIAA to enter the rat brain.

However, nothing is known about the effects of probenecid on the transport of 5-hydroxytryptophan or whether the higher concentration of 5-HIAA in probenecid-treated animals influences the turnover of 5-HT on raphé stimulation. Energy is also needed for uptake of 5-hydroxytryptophan into brain slices from the medium (McIlwain, 1966). Neff & Tozer (1968) pointed out that high concentration of 5-HIAA apparently does not influence turnover in normal animals, as the turnover of 5-HT is not altered when 5-HIAA increases after probenecid treatment. Although product inhibition of increased biosynthesis on nervous stimulation is suggested by our experiments, more evidence is needed to support this possibility.

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REFERENCES

AGHAJANIAN, G. K., ROSECRANS, J. A. & SHEARD, M. H. (1967). Serotonin : Release in the forebrain by stimulation of midbrain raphé. *Science, N.Y.*, **156**, 402–403.

ANDÉN, N.-E., CARLSSON, A., HILLARP, N.-Å. & MAGNUSSON, T. (1964). 5-Hydroxytryptamine release by nerve stimulation of the spinal cord. *Life Sci., Oxford*, **3**, 473–478.

ANDÉN, N.-E., CARLSSON, A., HILLARP, N.-Å. & MAGNUSSON, T. (1965). Noradrenaline release by nerve stimulation of the spinal cord. *Life Sci., Oxford*, **4**, 129–132.

ANDÉN, N.-E., DAHLSTRÖM, A., FUXE, K., LARSSON, K., OLSON, L. & UNGERSTEDT, U. (1966). Ascending monoamine neurons to the telencephalon and diencephalon. *Acta physiol. scand.*, **67**, 313–326.

ASHCROFT, G. W. & SHARMAN, D. F. (1962). Drug-induced changes in the concentration of 5-OR indolyl compounds in cerebrospinal fluid and caudate nucleus. *Br. J. Pharmac. Chemother.*, **19**, 153–160.

ASHCROFT, G. W., DOW, R. C. & MOIR, A. T. B. (1968). The active transport of 5-hydroxyindol-3-ylacetic acid and 3-methoxy-4-hydroxy-phenylacetic acid from a recirculatory perfusion system of the cerebral ventricles of the unanaesthetized dog. *J. Physiol., Lond.*, **199**, 397–425.

BOWERS, M. B., Jr. & GERBODE, F. (1968). CSF 5HIAA: Effects of probenecid and parachlorophenylalanine. *Life Sci., Oxford*, **7**, 773–776.

DAHLSTRÖM, A. & FUXE, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta physiol. scand.*, **62**, suppl. 232.

DESPOPOULOS, A. & WEISSBACH, H. (1957). Renal metabolism of 5-hydroxyindoleacetic acid. *Am. J. Physiol.*, **189**, 548–550.

ECCLESTON, D., PADJEN, A. & RANDIĆ, M. (1969). Release of 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid in the forebrain by stimulation of mid-brain raphé. *J. Physiol., Lond.*, **201**, 22–23*P*.

ECCLESTON, D., RANDIĆ, M., ROBERTS, M. H. T. & STRAUGHAN, D. W. (1969a). Release of amines and amine metabolites from brain by neural stimulation. In *Metabolism of Amines in the Brain*, ed. Hooper, G., pp. 29–33. London: Macmillan.

EULER, U. S. VON (1962). Problems in neurotransmission. In *Perspectives in Biology*, ed. Cori, C. F., Foglia, V. G., Leloir, L. F. & Ochoa, S., pp. 387–394. New York: American Elsevier.

GARATTINI, S., LAMESTA, L., MORTARI, A., PALMA, V. & VALZELLI, L. (1961). Pharmacological and biochemical effects of 5-hydroxytryptamine in adrenalectomized rats. *J. Pharm. Pharmac.*, **13**, 385–388.

GORDON, R., REID, J. V. O., SJOERDSMA, A. & UDENFRIEND, S. (1966). Increased synthesis of norepinephrine in the rat heart on electrical stimulation of the stellate ganglia. *Mol. Pharmac.*, **2**, 606–613.

HELLER, A., HARVEY, J. A. & MOORE, R. Y. (1962). A demonstration of a fall in brain serotonin following central nervous system lesion in the rat. *Biochem. Pharmac.*, **11**, 859–866.

HELLER, A. & MOORE, R. Y. (1965). Effect of central nervous system lesion on brain monoamines in the rat. *J. Pharmac. exp. Ther.*, **150**, 1–9.

KÖNIG, J. F. R. & KLIPPEL, R. A. (1963). *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* Baltimore: Williams and Wilkins Co.

KOSTOWSKI, W., GIACALONE, E., GARATTINI, S. & VALZELLI, L. (1969). Electrical stimulation of midbrain raphe: biochemical behavioral and bioelectrical effects. *Eur. J. Pharmac.*, **7**, 170–175.

MACINTOSH, F. C. & OBORIN, P. E. (1953). Release of acetylcholine from intact cerebral cortex. *Abstr. XIX Int. Physiol. Congr.*, pp. 580–581.

McILWAIN, H. (1966). *Biochemistry and the Central Nervous System*, 3rd ed., p. 131. London: Churchill.

NEFF, N. H., TOZER, T. N. & BRODIE, B. B. (1964). Indole metabolism. Part II. A specialized transport system to transfer 5-HIAA directly from brain to blood. *Pharmacologist*, **6**, 194.

NEFF, N. H., TOZER, T. N. & BRODIE, B. B. (1967). Application of steady-state kinetics to studies of the transfer of 5-hydroxyindoleacetic acid from brain to plasma. *J. Pharmac. exp. Ther.*, **158**, 214–218.

NEFF, N. H. & TOZER, T. N. (1968). In vivo measurement of brain serotonin turnover. *Adv. Pharmac.*, **6A**, 97–109.

OATES, J. A. (1961). Measurement of urinary tryptamine, tyramine and serotonin. *Meth. med. Res.*, **9**, 169–174.

PADJEN, A. & RANDIĆ, M. (1969). Release of 5-hydroxyindol-3-ylacetic acid in the forebrain by electrical stimulation of n. raphé. *Iugoslav. Physiol. Pharmac. Acta*, **5**, 349–353.

PFEIFER, K. A., VIZI, E. SZ., SATORY, E. & GALAMBOS, E. (1963). The effect of adrenalectomy on the norepinephrine and serotonin content of the brain and on reserpine action in rats. *Experientia*, **19**, 482–483.

RANDIĆ, M. & PADJEN, A. (1968). The release of 5-hydroxytryptamine in the brain by midbrain stimulation. *Iugoslav. Physiol. Pharmac. Acta*, **4**, suppl. 1, 215–220.

REID, W. D., VOLICER, L. & BRODIE, B. B. (1968). Inhibition of 5-hydroxyindoleacetic acid transport from the brain by 2,4-dinitrophenol. *Life Sci., Oxford*, **7**, 577–581.

RESNICK, R. H., SMITH, G. T. & GRAY, S. J. (1961). Endocrine influences on tissue serotonin content of the rat. *Am. J. Physiol.*, **201**, 571–573.

ROSECRANS, J. A., SHEARD, M. H. (1969). Effects of an acute stress on forebrain 5-hydroxytryptamine (5-HT) metabolism in C.N.S. lesioned and drug pretreated rats. *Eur. J. Pharmac.*, **6**, 197–199.

ROTH, R. H., STJÄRNE, L. & EULER, U. S. VON (1966). Acceleration of noradrenaline biosynthesis by nerve stimulation. *Life Sci., Oxford*, **5**, 1071–1075.

SEDVALL, G. C. & KOPIN, I. J. (1967). Acceleration of norepinephrine synthesis in the rat submaxillary gland in vivo during sympathetic nerve stimulation. *Life Sci., Oxford*, **6**, 45–51.

SHARMAN, D. F. (1966). Changes in the metabolism of 3,4-dihydroxyphenylethylamine (dopamine) in the striatum of the mouse induced by drugs. *Br. J. Pharmac. Chemother.*, **28**, 153–163.

SHEARD, M. H. & AGHAJANIAN, G. K. (1968). Stimulation of the midbrain raphe: Effect on serotonin metabolism. *J. Pharmac. exp. Ther.*, **163**, 424–430.

TOWNE, J. C. & SHERMAN, J. O. (1960). Failure of acute bilateral adrenalectomy to influence brain serotonin levels in the rat. *Proc. Soc. exp. Biol. N.Y.*, **104**, 721–722.

WEINER, N. & ALOUSI, A. (1966). Influence of nerve stimulation on rate of synthesis of norepinephrine. *Fedn Proc.*, **25**, 259.

WERDINIUS, B. (1967). Effect of probenecid on the levels of monoamine metabolites in the rat brain. *Acta pharmac. tox.*, **25**, 18–23.

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