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BRIEF COMMUNICATION

Release of 5-Hydroxytryptamine from Serotonergic Nerve Endings by α-Methyl-Meta-Tyramine

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MCBRIDE, W. J. AND M. H. APRISON. *Release of 5-hydroxytryptamine from serotonergic nerve endings by α-methyl-meta-tyramine.* PHARMAC. BIOCHEM. BEHAV. 1(5) 587–590, 1973.–Preparations of synaptosomes (P_2) from the telencephalon of the pigeon and rat were used to study the effects of α-methyl-*m*-tyramine (α-MMTA) and metaraminol on the efflux of radioactive serotonin (5-HT). The preparations were first incubated in the presence of 2.5×10^{-8} M [^{3}H]-5-HT to selectively label serotonergic nerve endings. Both α-MMTA and metaraminol in combination at a concentration of 0.15, 0.030 and 0.006 mM each significantly increased the efflux of [^{3}H]-5-HT over control values in preparations from both the pigeon and rat. These two compounds together at the two higher concentrations also appeared to decrease the efflux of [^{3}H]-5-hydroxyindoleacetic acid. At a concentration of 0.012 mM, α-MMTA significantly increased the efflux of [^{3}H]-5-HT whereas metaraminol when tested alone at this concentration did not appear to have an effect on the release of [^{3}H]-5-HT. The results are discussed in terms of the behavioral effects produced by α-methyl-meta-tyrosine in pigeons working on a multiple FR 50 FI 10 schedule of reinforcement.

Serotonin release α-Methyl-*m*-tyramine Metaraminol Synaptosomes 5-Hydroxyindoleacetic acid release

IT HAS been reported [1] that intramuscular injections of 100 mg/kg of α-methyl-meta-tyrosine (αMMT) resulted in decreases in total 5-hydroxytryptamine (serotonin or 5-HT) levels in brain which were temporally related to behavioral depression in pigeons trained to work for food on a multiple fixed-ratio 50, fixed-interval 10 (Mult FR 50 FI 10) schedule of reinforcement. Although the levels of norepinephrine and 3,4-dihydroxyphenylethylamine also decreased in these brains, the fall in content of these two compounds did not show a temporal correlation with behavioral disruption. Injections of α-MMT have also been found to disrupt the behavior of rats working on a variable interval schedule of food reinforcement (Hingtgen and Aprison, unpublished). In an attempt to explain the cause of the disruption of the behavior, Aprison and Hingtgen [1,2] suggested that, although the total 5-HT content in brain fell, the behavioral effect seen after the administration of α-MMT may be due to an increased release of 5-HT into the synaptic cleft of specific synapses. To test this hypothesis, the effects of α-MMT on preparations of synaptosomes isolated from the telencephalon of the pigeon were studied [9]. This report established that α-MMT itself had no apparent effect on either the uptake or release of 5-HT from isolated synaptosomal preparations but that two metabolites of α-MMT, namely α-methyl-meta-tyramine (α-MMTA) and metaraminol, either individually or in combination, increased the release of labelled 5-HT. However, with the incubation conditions used in these latter studies, it was not possible to ascertain whether the 5-HT was released from serotonergic or nonserotonergic nerve endings or from both types. Since these studies [9] were completed, another report has been published which suggests that under the proper incubation conditions the serotonergic nerve endings can be selectively labelled with radioactive serotonin [7]. Therefore, the present experiments were designed to determine if α-MMTA and metaraminol could exert an action on serotonergic nerve endings.

METHOD

Naive, white Carneaux cocks, 8–9 months old and weighing approximately 0.5 kg, and female Wistar rats, weighing approximately 200 g, were used in these studies. The telencephalon was used for the preparation of the crude synaptosomal fraction (P_2) according to a procedure based on that of Gray and Whittaker [5] as previously described [9].

Incubation Conditions

In these studies, the crude synaptosomal fraction (P_2) was used for all incubations. This fraction was resuspended in standard bathing medium to give a protein concentration of approximately 2 mg/ml. This medium is similar to that previously described [10] and consisted of (in mM): 120 NaCl, 4.75 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 0.75 $CaCl_2$, 25 $NaHCO_3$ and 10 D-glucose in equilibrium with 95% O_2 - 5% CO_2 (note that in the present studies $MgSO_4$ was used in place of $MgCl_2$); 2×10^{-5} M nialamide was also present in the medium during all the incubation and washing procedures. This concentration of nialamide inhibited 95% of the MAO activity of the extrasynaptosomal mitochondria [9].

The crude synaptosomal fraction was incubated for 5 min prior to the addition of 0.20 μCi of [^{3}H]-5-hydroxytryptamine (specific radioactivity 6.8 Ci/mmol; Amersham-Searle; Arlington Heights, Ill.) to give a final concentration of 2.5×10^{-8} M in 1.20 ml of incubation medium. Suspensions were then incubated for 10 min at 37°C under a continous stream of 95% O_2 - 5% CO_2. Incubations were terminated by first placing the test tubes (13 x 100 mm) in crushed ice and then centrifuging for 2 min at 2,500 rev/min (1200 x g) in a refrigerated centrifuge (International Equipment Co., Model PR-2, Needham Heights, Mass.). After the supernatant was decanted, the pellet was resuspended in 2 ml of ice-cold glucose-bicarbonate-saline medium containing nialamide (2×10^{-5} M) and immediately centrifuged again at 2,500 rev/min for 2 min. The resulting pellet was used for efflux studies.

The procedure used for studying the release was similar to that previously described [9] except that the procedure of Kariya and Aprison [6] was used to isolate 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) for subsequent radioactivity measurements. This latter procedure proved to be superior to that used in the previous study; more samples could be analyzed in less time with less effort and with a higher degree of reproducibility. Briefly, aliquots (0.20 ml) of the suspension were withdrawn at "0" and "5" minute time points and immediately centrifuged; a 0.150 ml portion of the resulting supernatant was added to a 3 ml test tube (in crushed ice) containing 0.250 ml of 0.20 M NaH_2PO_4/K_2HPO_4 buffer, pH 6.1. A 0.300 ml portion was then transferred to an ion-exchange column (0.4 x 1.0 cm; Bio-Rex 70, 200–400 mesh; Bio-Rad Laboratories, Richmond, California). When the level of the liquid had completely entered the resin, 1 ml of 0.02 M, pH 6.1, phosphate buffer was added. This first 1.3 ml were collected in one tube and contained [^{3}H]-5-HIAA. The [^{3}H]-5-HT is then eluted with 2.0 ml of 1N HCl and is collected in a second tube. A 0.50 ml portion is withdrawn from each tube and transferred to polyethylene scintillation vials. The

TABLE 1

EFFECTS OF α-METHYL-META-TYRAMINE AND METARAMINOL ON THE EFFLUX OF [^{3}H]-5-HYDROXYTRYPTAMINE AND [^{3}H]-5-HYDROXYINDOLEACETIC ACID FROM SYNAPTOSOMES (P_2) ISOLATED FROM THE TELENCEPHALON OF THE PIGEON

Incubation Conditions	% of total released in 5 min	
	5-HT	5-HIAA
1. Control	1.86 ± 1.26	14.8 ± 1.12
+ 0.15 mM α-MMTA and 0.15 mM metaraminol	24.2 ± 2.71‡	10.8 ± 0.70‡
2. Control	1.60 ± 1.34	15.7 ± 0.78
+ 0.030 mM α-MMTA and 0.030 mM metaraminol	18.3 ± 3.46‡	13.5 ± 1.59*
3. Control	2.49 ± 0.67	15.6 ± 0.58
+ 0.006 mM α-MMTA and 0.006 mM metaraminol	5.67 ± 1.44†	15.6 ± 1.45
4. Control	2.98 ± 0.25	14.3 ± 1.23
+ 0.012 mM α-MMTA	10.7 ± 1.50‡	13.5 ± 1.55
5. Control	3.05 ± 1.49	16.0 ± 2.70
+ 0.012 mM metaraminol	3.78 ± 1.49	21.2 ± 1.28*

The total amount of radioactivity taken up by P_2 was in the range (±10%) of 85,000 d.p.m./mg protein. The data represent the means ± S.D. of 4 determinations. Statistical significance was determined with the student's *t*-test. Significance of difference between control and drug treated preparations are as follows: *$p<0.05$; †$p<0.01$; ‡$p<0.005$.

1N HCl fraction was then neutralized with NaOH and 15 ml of Bray's solution [4] were added. To determine the total amount of radioactivity present, 0.150 ml of the suspension was added directly to 0.250 ml of the 0.20 M phosphate buffer containing 3% Triton X-100. A 0.200 ml portion was used for counting purposes. Of the total amount of radioactivity in the final suspension added to the column, 91 ± 4% was recovered in the 5-HT and 5-HIAA fractions. Preliminary experiments indicated that at the "0" time point, there was approximately the same amount of radioactivity in the 5-HT pool as was in the 5-HIAA pool in the P_2 fraction of the rat.

All radioactivity measurements were made with a scintillation counter (Packard Tricarb, Model 3375, Packard Instrument Co., Inc., Downers Grove, Ill.) equipped with automatic external standardization to monitor counting efficiency.

RESULTS

Under control conditions very little [^{3}H]-5-HT is released from the incubated crude synaptosomal fraction (P_2) isolated from the telenchephalon of the rat or pigeon (Tables 1 and 2). Treatment of the P_2 fraction isolated from the telencephalon of the pigeon with α-MMTA and metaraminol resulted in a significant increase in the release of [^{3}H]-5-HT (Table 1). When these two amines were tested in combination at a concentration of 0.15, 0.030 and 0.006 mM each, the efflux of [^{3}H]-5-HT increased 13, 11 and 2.3 fold, respectively, over control values. In addition, at the two higher concentrations tested, there appeared to be a small reduction in the efflux of [^{3}H]-5-HIAA. This small reduction may be a reflection of the lower amounts of [^{3}H]-5-HT present in synaptosomal preparations treated with drugs; since there is less [^{3}H]-5-HT present for monoamine oxidase to act on then less [^{3}H]-5-HIAA is produced and will be available for release. In the case of the P_2 fraction isolated from the telencephalon of the rat, α-MMTA and metaraminol when tested together also markedly increased the efflux of [^{3}H]-5-HT and appeared to decrease the release of [^{3}H]-5-HIAA (Table 2). These two metabolites of α-methyl-meta-tyrosine, when used in combination at a concentration of 0.15, 0.030 and 0.006 mM each, increased the release of [^{3}H]-5-HT over control values by 11, 7 and 3.5 fold, respectively.

When α-MMTA and metaraminol were tested individually at a concentration of 0.012 mM on synaptosomal preparations from the pigeon and rat, α-MMTA significantly increased the release of [^{3}H]-5-HT three-fold while metaraminol had no apparent affect on its release (Tables 1 and 2). However, in those experiments where only metaraminol was tested there appeared to be a slightly greater release of [^{3}H]-5-HIAA (Tables 1 and 2).

TABLE 2

EFFECTS OF α-METHYL-META-TYRAMINE AND METARAMINOL ON THE EFFLUX OF [^{3}H]-5-HYDROXYTRYPTAMINE AND [^{3}H]-5-HYDROXYINDOLEACETIC ACID FROM SYNAPTOSOMES (P_2) ISOLATED FROM THE TELENCEPHALON OF THE RAT

Incubation Conditions	% of total released in 5 min 5-HT	5-HIAA
1. Control	3.60 ± 1.41	14.9 ± 1.61 (3)
+ 0.15 mM α-MMTA and 0.15 mM metaraminol	41.3 ± 2.70‡	8.21 ± 2.62*
2. Control	2.17 ± 1.65	14.2 ± 0.67
+ 0.030 mM α-MMTA and 0.030 mM metaraminol	15.2 ± 5.23 (3)‡	12.6 ± 0.47†
3. Control	2.96 ± 2.75	13.9 ± 0.60
+ 0.006 mM α-MMTA and 0.006 mM metaraminol	10.4 ± 1.13‡	11.6 ± 0.73†
4. Control	3.05 ± 2.15	11.9 ± 0.78
+ 0.012 mM α-MMTA	8.71 ± 0.63‡	12.8 ± 0.70
5. Control	4.96 ± 0.29	10.9 ± 0.64
+ 0.012 mM metaraminol	5.36 ± 1.71	13.0 ± 0.52‡

The total amount of radioactivity taken up was in the range (±10%) of 75,000 d.p.m./mg protein. The data represent the means ± S.D. of four determinations unless otherwise noted. Significance of difference between control and drug treated preparations are as follows: *$p<0.05$; †$p<0.01$; ‡$p<0.005$.

DISCUSSION

The present findings suggest that α-MMTA, in low concentrations, can increase the efflux of [^{3}H]-5-HT from preparations of synaptosomes (P_2) isolated from the telencephalon of the pigeon and rat. This finding is in agreement with that previously published [9] for the pigeon where much higher drug concentrations were used. In that study [9], both metaraminol and α-MMTA, when tested individually at a concentration of 1.5 mM, appeared to be equally effective in increasing the efflux of labelled 5-HT. Since it is likely that both of these metabolites of α-methyl-*m*-tyrosine are present *in vivo* and since it appeared that both were equally effective in increasing the release of 5-HT they were tested mainly in combination. The finding that in one study [9] metaraminol appeared to increase the release of 5-HT while in the present study it did not appear to have any affect on the efflux of 5-HT could be explained on the following basis. In our previous study, the concentration of radioactive 5-HT used to label the synaptosomal pool was 5×10^{-6} M. At this concentration, it has been suggested that 5-HT may enter nerve terminals (e.g., noradrenergic nerve terminals) other than serotonergic ones [7,8]. Therefore, the combination of using a higher drug concentration and labelling of different populations of nerve endings could explain the apparent effectiveness of metaraminol in increasing the release of 5-HT. The release observed in the previous study may not have been predominantly from serotonergic nerve endings.

Our previous study demonstrated that, after subfractionation of the incubated P_2 fraction by standard procedures [5], at least 84% of the radioactive 5-HT taken up was distributed in the region of the discontinuous sucrose gradient containing the nerve endings. Therefore, on the basis of the data obtained in our previous study [9] and that obtained from our present experiments, it appears that serotonin can be selectively released from serotonergic nerve endings by α-MMTA. These data support the hypothesis of Aprison and Hingtgen [1,3] to explain the behavioral effects they observed with pigeons given intramuscular injections of α-methyl-*m*-tyrosine, namely, that the temporal relationship which exists between behavioral depression and lower 5-HT levels can be explained on the basis of increased levels of 5-HT in the synaptic cleft of serotonergic synapses. Since the time course of the concentrations of α-methyl-*m*-tyramines in brain varied inversely with respect to the concentration of 5-HT (as well as of norepinephrine and dopamine), the present studies suggest that the lower levels of 5-HT found in the brains of pigeons in these behavior studies may be due in part to the release of 5-HT from nerve terminals by α-MMTA, a major metabolite of α-methyl-*m*-tyrosine.

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