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BRIEF COMMUNICATION

Effect of Imipramine on Serotonin Turnover in the Lateral Hypothalamus[1]

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KANTAK, K. M., M. J. WAYNER, J. M. STEIN AND H. A. TILSON. *Effect of imipramine on serotonin turnover in the lateral hypothalamus.* PHARMAC. BIOCHEM. BEHAV. 9(5) 693–696, 1978.—One hour following an infusion of ^{3}H-5-hydroxytryptamine, animals were injected with either 15 mg/kg imipramine hydrochloride or 0.9% NaCl and then the lateral hypothalamus was perfused for 40 min. Samples of perfusate were analyzed by thin layer chromatography for estimation of ^{3}H-labelled 5-hydroxytryptamine and metabolites. The results indicate that impramine hydrochloride 15 mg/kg decreases serotonin release and turnover in the lateral hypothalamus.

Serotonin turnover Imipramine hydrochloride Push-pull perfusion 5-Hydroxytryptamine
Lateral hypothalamus Serotonin release

IMIPRAMINE is a tricyclic antidepressant drug which can prevent the reuptake of 5-hydroxytryptamine (5-HT) in the brain [12]. The effects of imipramine on 5-HT metabolism have been investigated. Brain concentrations of 5-HT either remain unchanged or increase after imipramine treatment [2,5]. Imipramine also increases whole brain ^{3}H-5-HT formed from ^{3}H-L-tryptophan [13]. During push-pull perfusion of the lateral cerebroventricle, chlorimipramine [6], and imipramine [9] increase the efflux of ^{3}H-5-HT. Following chlorimipramine measurements of ^{3}H-5-HIAA were not made; whereas, following imipramine measurements were made but differences between the drug and control conditions were not found. However, whole brain 5-HIAA decreases after imipramine administration [2,5]. The fact that imipramine does not affect ^{3}H-5-HIAA in the lateral cerebroventricle might be related to accumulation of 5-HIAA by the choroid plexus [3] and thereby obscures any drug induced changes. The purpose of the present investigation was to measure directly the effect of imipramine on the efflux of ^{3}H-5-HT and ^{3}H-5-HIAA during push-pull perfusion in brain tissue. The lateral hypothalamus was selected because changes in ^{3}H-5-HT metabolism during push-pull perfusion have been reported to occur in this brain region [7, 8, 10].

METHOD

Animals

Six male hooded rats, 400–517 g, from our colony were utilized. Animals were housed in individual living cages. They had free access to Purina lab chow blocks and water. Animals were kept on a constant light/dark cycle. The 12 hr light phase began at 0600 hr and was followed by a 12 hr dark phase. The room temperature was maintained at 21° ± 1°C.

Surgery and Histology

Surgery was performed under Equi-Thesin anaesthesia (Jensen-Salsbery Laboratories) at a dose of 3 cc/kg. Each animal was implanted with a concentric push-pull cannula 0.5 mm above the right lateral hypothalamus according to predetermined DeGroot [4] coordinates: AP 5.4, L 1.8 and V 3.0 mm from the interaural line. The inner cannula extended 0.5 mm beyond the end of the implanted outer cannula. There were 2 weeks of post-operative recovery prior to the start of the experiment.

When the experimental conditions were terminated all animals were perfused intracardially, first with 0.9% NaCl, and then with neutralized 10% Formalin plus 0.9% NaCl. The

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brains were removed, frozen, and sectioned at 60 μ. Tissue was stained with cresyl violet and examined to determine the location of the cannula tip.

Apparatus

The perfusion chamber consisted of 20×20×50 cm Plexiglas box with a standard stainless steel rod grid floor and enclosed in a lighted sound attenuating chamber fitted with an exhaust fan. All push-pull cannulae perfusions were performed with a Sage Instruments Model 375 A tubing pump. All radioactive determinations were made with a Beckman LS 100 C Scintillation Counter.

Drugs

Imipramine hydrochloride, 15 mg, was dissolved in 1 ml of isotonic NaCl. Isotonic NaCl was used for the vehicle control. All injection volumes were 1 ml/kg and were injected intraperitoneally.

Procedure

Animals were infused, via the push-pull cannula, with 0.5 μCi (13 ng) of ^{3}H-5-hydroxytryptamine binoxalate (specific activity = 5.7 Ci/m mole, New England Nuclear) 1 hr prior to push-pull perfusion. The procedure utilized a Harvard infusion pump and 0.5 μl was infused at a rate of 1 μl/min. Three min before the start of the perfusion animals were injected with either 15 mg/kg imipramine hydrochloride (n=3) or 0.9% NaCl (n=3). Animals were perfused at a rate of approximately 20 μl/min for 40 min with 0.9% bacteriostatic saline (Eli Lilly & Co). Eight 5 min samples of perfusate were collected in vials containing 0.1 ml of 1.0 N formic acid. A 20 μl aliquot was taken for each sample and analyzed for total radioactive content. In addition Samples 4, 5 and 6 were further analysed by thin layer chromatography (TLC) for estimation of ^{3}H-labelled 5-HT, 5-HIAA, 5-methoxytryptamine (5-MT), 5-methoxytryptolphol (5-MTPhol), and 5-methoxyindoleacetic acid (5-MIAA). Detailed descriptions of these procedures are reported elsewhere [7, 8, 10]. All cpm were corrected for background, efficiency, dilution with the formic acid, and recovery.

RESULTS

Histology

The tips of all cannulae were in the lateral hypothalamus, lateral to the fornix, and medial to the cerebral peduncle. All placements were within the anterior-posterior limits of the lateral hypothalamus according to the DeGroot atlas, 5.8–5.0.

Aliquot Analysis

Total radioactivity collected from the 8 samples was analyzed by a 2×8 analysis of variance with repeated measures [17]. The factors were the 2 treatments and the 8 samples. The μ Ci/5 min sample for the imipramine and control treatments are presented in Fig. 1. There were no significant differences in the μCi/5 min for the treatment main effect. As would be expected, there was a significant main effect for samples, $F(7,28)=10.05$, $p<0.01$. Tukey A tests of the differences within and between groups for Samples 4, 5 and 6 revealed no significant differences. Therefore, the efflux of radioactivity was similar in the samples upon which the TLC analyses were performed. Although the treatment main effect was not significant, the efflux of radioactivity was not similar for both groups because there was a significant interaction effect, $F(7,28)=3.36$, $p<0.01$. Further testing with Dunnett's tests revealed significantly less total radioactivity in Samples 1 and 2 for the imipramine group compared to the control group, $p<0.01$ and 0.05 respectively.

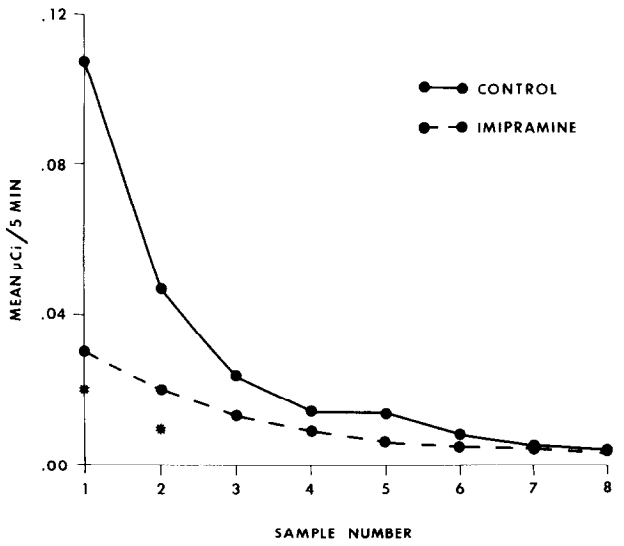

FIG. 1. Mean μCi/5 min sample of total radioactivity. ●——● 0.9% NaCl and ●– – –● Imipramine hydrochloride 15 mg/kg. *Significantly different from the control.

TLC Analysis

Figure 2 represents the mean n Ci/5 min of ^{3}H-5-HT and ^{3}H-mebatolites detected in the perfusate after bidirectional TLC separation of Samples 4, 5 and 6. Individual t-tests were performed, comparing the mean nCi/5 min in the control treatment with the imipramine treatment. In animals treated with imipramine hydrochloride 15 mg/kg, there were significantly less nCi/5 min for 5-HIAA, $t(4)=8.46$, $p<0.01$, and 5-MTPhol, $t(4)=7.93$, $p<0.01$. All other comparisons were not significant.

^{3}H-5-HT standard plates were prepared with each perfusion to check for non-specific spread of radioactivity. On these plates less than 5% of the total radioactivity was non-specific with respect to the ^{3}H-5-HT spot for each group. In contrast, the metabolites of 5-HT from the perfusate represented 42% and 12% of the total radioactivity per 5 min for the control and imipramine groups respectively. Thus the radioactivity attributable to the metabolites in the perfusate represents functional metabolism and not just an artifact due to non-specific radioactive spread on the TLC plate.

DISCUSSION

The results from this experiment demonstrate that imipramine hydrochloride 15 mg/kg decreases the turnover of 5-HT. Following imipramine administration decreases in the formation of 5-HIAA and 5-MTPhol in lateral hypothalamic perfusate were found. 5-MTPhol is an alcohol metabolite of 5-MT which is directly metabolized from 5-HT [1,11]. There were no changes in 5-HT. These results are in agreement with whole brain tissue studies which report no changes in 5-HT level but decreases in 5-HT turnover following imip-

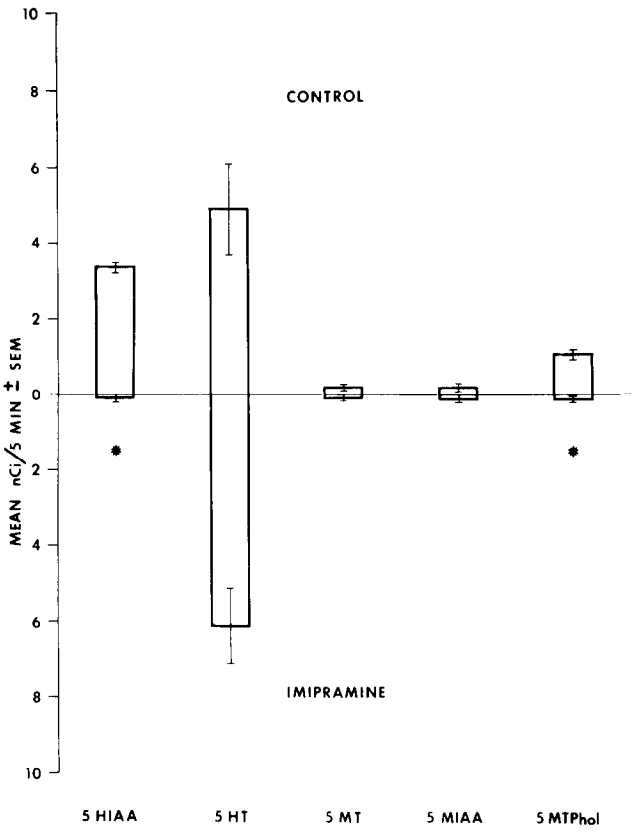

FIG. 2. Mean nCi/5 min sample of ^{3}H-5-HIAA, ^{3}H-5-HT, ^{3}H-5-MT, ^{3}H-5-MIAA and ^{3}H-5-MTPhol detected in Samples 4, 5 and 6. The top half of the figure is the 0.9% NaCl control group and the bottom half of the figure is the 15 mg/kg imipramine hydrochloride group. *Significantly different from the control.

ramine administration [5]. In contrast ventricular perfusion yields increases in 5-HT [6,9] with no detectable changes in 5-HT turnover [9] following reuptake inhibition. Therefore, the site of push-pull perfusion might be differentially sensitive to imipramine's action on 5-HT metabolism.

In addition to the above changes, significant decreases in the efflux of total radioactivity during the first 10 min of push-pull perfusion occurred in the imipramine treated animals. Since imipramine slows the firing rate of midbrain raphe units within 3–5 min after intraperitoneal administration [15] and release of 5-HT is nerve impulse dependent [14], a decreased release of 5-HT following imipramine treatment is indicated. Following the first 10 min of push-pull perfusion, no further differences in the efflux of total radioactivity were found between groups which can probably be related to the rapid decline in total radioactivity in the control group during the first 15–20 min. Under normal conditions a rapid decline of radioactivity is characteristic of radioactive washout [16]. Following the rapid declining phase which can endure from 10 to 30 min, a smaller and more stable efflux of radioactivity occurs. In other push-pull perfusion studies in which reuptake inhibitors were administered, no differences in total radioactivity were found between groups [6,9]. However, the drugs were injected 40–60 min following the start of the push-pull perfusion. At a time when efflux of radioactivity is small and stable, it is difficult to detect differences. The present data reflect changes in release rather than uptake of ^{3}H-5-HT because imipramine was administered almost 1 hr following the infusion of ^{3}H-5-HT. If imipramine were administered prior to the infusion, then one would expect to detect a difference in 5-HT uptake. In this case, imipramine pretreated animals would produce more total radioactivity in the washout because the membrane pump which transports 5-HT intraneuronally would be inhibited. Therefore, a comparison of results from animals in which the drug has been administered prior to the labelled 5-HT with animals in which the 5-HT was infused prior to the imipramine will provide specific information on drug induced changes in uptake or release of the neurotransmitter as well as its metabolism during push-pull perfusion.

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