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The Effects of Serotonin Depletion on the Voltammetric Response to Amphetamine

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HASKETT, C AND K MUELLER *The effects of serotonin depletion on the voltammetric response to amphetamine* PHARMACOL BIOCHEM BEHAV **28**(3) 381–384, 1987 —In vivo voltammetry with carbon paste electrodes reliably produces two oxidation peaks Previous research suggests that in caudate peak 1 (P1) monitors ascorbic acid and peak 2 (P2) monitors uric acid To provide additional evidence that P2 monitors uric acid rather than indoles, the effects of the serotonin synthesis inhibitor p-chlorophenylalanine (PCPA) were studied in caudate (serotonin-poor) and globus pallidus (serotonin-rich) In both caudate and globus pallidus PCPA had little effect on P2 and pretreatment with PCPA failed to inhibit the amphetamine-induced increase in P2 In general, P2 recorded from globus pallidus was always very similar to P2 recorded from caudate These data are consistent with the hypothesis that P2 represents uric acid even in serotonin-rich areas of the brain Pretreatment with PCPA dramatically enhanced the amphetamine-induced increase in P1 in caudate but not in globus pallidus This finding is interesting in light of reports that PCPA enhances certain behavioral effects of amphetamine

In vivo voltammetry   Ascorbic acid   Uric acid   p-Chlorophenylalanine   Amphetamine   Caudate   Globus pallidus

IN vivo voltammetry monitors the release of electroactive substances (such as dopamine and its metabolites, ascorbic acid, uric acid, serotonin and its metabolites, etc) into the extracellular space of the brain However, a controversy has evolved around the ability of voltammetry to distinguish between catecholamines and ascorbic acid (AA), and to distinguish between indoles and uric acid (UA) In order to provide additional evidence that UA is monitored by carbon paste electrodes, the voltammetric effects of p-chlorophenylalanine (PCPA), a serotonin (5HT) synthesis inhibitor, were studied in caudate and globus pallidus

Since its development by Kissinger, Hart and Adams in 1973 [9], voltammetry has often been used to monitor DA [3, 9, 18] and 5HT [8,12] release. However, the "catecholamine" signal produced by carbon paste electrodes has recently been attributed to AA [2,14] This signal is increased by amphetamine but appears to reflect AA rather than catecholamines [6,17]. Recent studies microinfusing UA, uricase, allopurinol and xanthine oxidase have likewise suggested that UA confounds the signal once thought to be entirely due to indoles [4,15] Data from striatal carbon paste electrodes have suggested that virtually the entire "indole" signal is contributed by UA [15]. However, the striatum is relatively 5HT-poor, carbon paste electrodes might detect 5HT or other indoles in a 5HT-rich area of the brain.

In order to provide additional evidence that UA is the primary contributor to P2, even in a 5HT-rich area of the brain, carbon paste electrodes were implanted in both caudate and globus pallidus. Globus pallidus contains three times as much serotonin as the anterior caudate and about as much 5HIAA as the raphe [13] 5HT levels were depleted with PCPA, a well known tryptophan hydroxylase inhibitor. PCPA produces relatively rapid and long-lasting (>4 days) depletion of 5HT and 5HIAA throughout rat brain. In forebrain areas one can expect more than 70% depletion of both 5HT and 5HIAA [5, 7, 10].

If UA is the primary contributor to P2 in globus pallidus, as it is in caudate, the signal recorded from globus pallidus should respond in the same manner as the signal recorded from caudate However, if P2 decreases in globus pallidus after pretreatment with PCPA, and if PCPA inhibits the amphetamine-induced increase in P2 in globus pallidus, one could conclude that indoles contribute to P2 in globus pallidus

METHOD

Fourteen male albino rats (mean body weight=390 g) were housed singly with free access to food and water A 12 hour light-dark cycle was maintained.

Carbon paste working electrodes were constructed by pulling the teflon sheath over the end of a silver wire (Medwire, Ag 10T) and filling the resulting cavity with carbon paste as previously described [15] Two working electrodes were placed in the anterior caudate nucleus at 2 8 mm anterior to bregma, 2.8 mm lateral, 5.2 mm beneath the cortex, two electrodes were placed in the globus pallidus at 0 8 mm anterior to bregma, 3.5 mm lateral, 6 5 mm beneath the cortex [16]. A silver wire attached to a skullscrew served as an auxiliary electrode. A reference electrode was constructed from a silver wire anodized in 0.1 M HCl and placed

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TABLE 1

TESTING AND INJECTION SCHEDULE

	Day 0	Day 1	Day 2*	Day 3	Day 4	Day 5
Group PCPA	Surgery	Recovery	PCPA	PCPA	No Drug	Amphetamine
Group SAL	Surgery	Recovery	Saline	Saline	No Drug	Amphetamine

*Recording was conducted on days 2, 3, 4 and 5

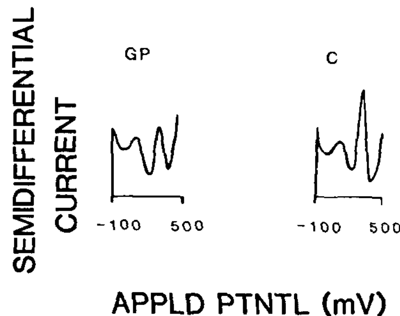

FIG 1 Preinjection scans recorded from caudate (C) and globus pallidus (GP) of the same animal Even though globus pallidus contains far more 5HT and 5HIAA than caudate, there is no consistent difference in the relative heights of P2

in a disposable pipette tip filled with 3 M NaCl in 10% gelatin

The animals were allowed 48 hours to recover from the surgical procedure After this recovery period, voltammetric testing began each day 2 hours after lights-on In order to ensure a stable baseline, recording was always conducted for 2 hours before any drug was administered A BAS DCV-5 voltammetry controller remotely controlled by an Apple II+ microcomputer was used to conduct semidifferential linear sweep voltammetry. The applied potential increased from −100 mV to 500 mV at 10 mV per second Electrodes were scanned every 12 min.

The testing schedule is summarized in Table 1 On the second and third days after surgery, Group PCPA (n=7) was injected with 250 mg/kg PCPA methyl ester (Sigma), intraperitoneally; Group SAL (n=7) was injected with an equivalent amount of normal saline. PCPA produces a transient reduction in catecholamines but catecholamine levels return to normal within 24 hours [7,10] Therefore, the day following PCPA, recording was conducted for 2 hours but no drug was administered. The fifth day after surgery, all animals were injected with 3 mg/kg d-amphetamine sulfate (subcutaneously) Electrode positions were verified postmortem by examination of frozen sections Data obtained from animals with misplaced electrodes were discarded.

The results are presented as percent deviation from the preinjection baseline (baseline is defined as the mean peak height of the three scans prior to injection) The data were analyzed with a 2-way mixed effects ANOVA, electrode location is the within-subjects variable and drug (PCPA) pretreatment is the between-subjects variable

RESULTS

Two oxidation peaks were observed· peak 1 (P1), the putative AA peak, and peak 2 (P2), the putative UA peak As shown in Fig 1, there were no obvious differences between the voltammograms recorded from caudate or globus pallidus even though the two areas of the brain have dramatically different amounts of 5HT and 5HIAA.

Figure 2 shows the effects of PCPA on P1 and P2 on the first day of administration (the second day after surgery). In general, PCPA produced a small non-significant decrease in P2 Again, the PCPA data from caudate and globus pallidus are virtually identical even though globus pallidus contains far more 5HT than caudate. Data from the second day of PCPA treatment (third day after surgery) are not shown, the effects of PCPA were statistically non-significant and small (<5% change) in magnitude

To further assess the effects of PCPA, the pre-injection baselines of the daily recording sessions were compared at 2 hr prior to PCPA and 24, 48 and 72 hr after PCPA These data should reflect the reduction of 5HT and 5HIAA which occurs over a period of days However, there were no statistically significant differences in P1 or P2 due to electrode location or drug (PCPA) at anytime

As expected, amphetamine caused an increase in P1 in caudate, it also increased P1 in globus pallidus (see Fig 3) In general, the amphetamine-induced increase in P1 was greater in caudate than in globus pallidus at the first and second hour after injection, $F(1,10)=22.93$, $p<0.01$, hr 1, $F(1,10)=9.85$, $p<0.05$, hr 2; however, this difference became non-significant at the third hour ($p>0.05$) Inspection of the figure suggests that this difference is due primarily to the PCPA-caudate group Pretreatment with PCPA dramatically enhanced the amphetamine-induced increase in P1 in caudate, but not in globus pallidus, that is, the interaction (electrode location × drug pretreatment) was significant, $F(1,10)=21.14$, $p<0.01$, hr 1, $F(1,10)=11.67$, $p<0.01$, hr 2, $F(1,10)=7.44$, $p<0.025$, hr 3

In general, amphetamine produced an increase in P2 (see Fig 3) Pretreatment with PCPA failed to inhibit the amphetamine-induced increase in P2, in fact, P2 appeared to be enhanced by pretreatment with PCPA but this effect did not reach statistical significance In general, the increase in P2 tended to be somewhat higher in caudate than in globus pallidus, but the main effect of electrode location was only significant at the third hour, $F(1,10)=0.83$, $p>0.05$, hr 1, $F(1,10)=0.003$, $p>0.05$, hr 2, $F(1,10)=7.17$, $p<0.05$, hr 3 Pretreatment with PCPA slightly enhanced the amphetamine-induced increase in P2 in caudate but not in globus pallidus, $F(1,10)=5.12$, $p<0.05$, hr 1, however, this interaction was non-significant at 2 and 3 hours ($p>0.05$)

DISCUSSION

The purpose of this research was to examine the effects of 5HT depletion on P2 recorded by carbon paste electrodes in

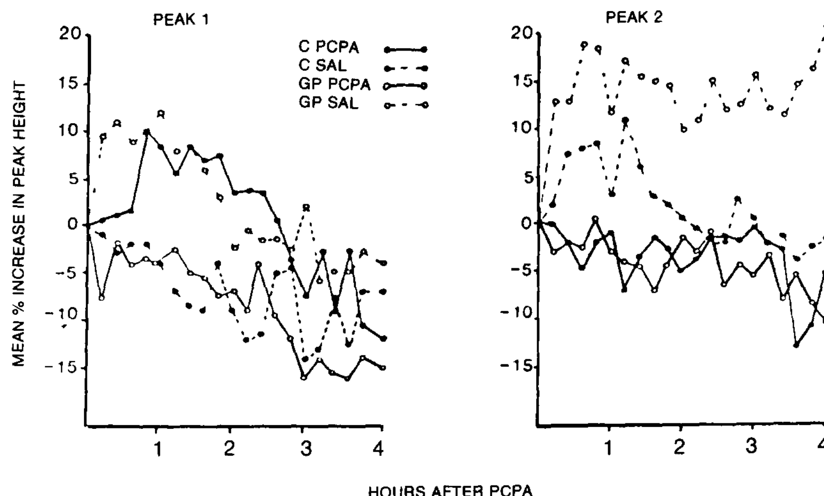

FIG 2 The effect of PCPA (250 mg/kg, IP) on voltammetric peaks 1 (AA) and 2 (UA) in the caudate and globus pallidus Mean percent increase from baseline (recording prior to administration of drug) is shown *Indicates statistically significant differences between means (ANOVA)

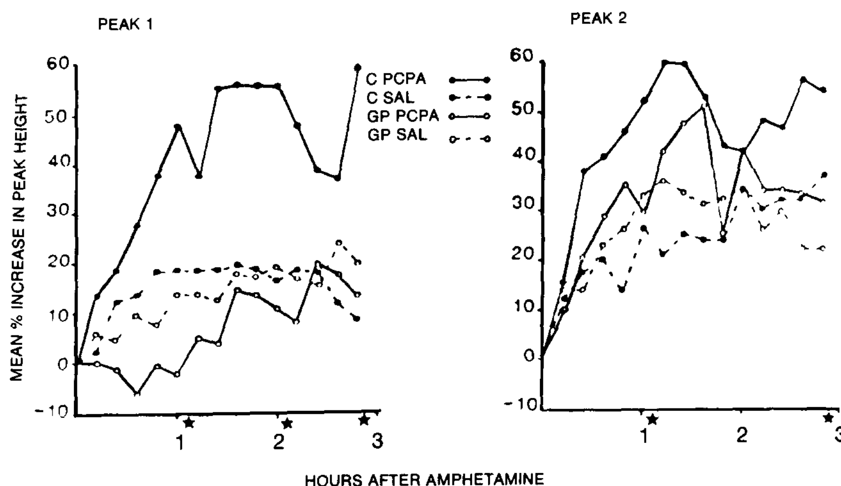

FIG 3 The effect of d-amphetamine (3 mg/kg, SC) on voltammetric peaks 1 (AA) and 2 (UA) in the rat caudate and globus pallidus Mean percent increase from baseline (recording prior to administration of drug) is shown *Indicates statistically significant differences between means (ANOVA) To avoid alpha errors, data were analyzed only at 1, 2, and 3 hours after injection

globus pallidus and caudate. PCPA had little effect on P2, the putative UA peak, in both caudate and globus pallidus at anytime during the experiment These data are consistent with the hypothesis that P2 represents UA even in globus pallidus, a 5HT-rich area of the brain An unexpected finding was that pretreatment with PCPA enhanced the amphetamine-induced increase in P1 in caudate.

Since P2 in caudate has previously been shown to monitor UA rather than indoles [15], the lack of effect of PCPA on P2 recorded from caudate during the 4 hr after injection was expected P2 recorded from globus pallidus responded similarly to P2 in caudate In addition PCPA had no significant effect on P2 at 24, 48, or 72 hours after PCPA in either caudate or globus pallidus These data were expected in caudate, P2 recorded from globus pallidus responded in the same way as P2 recorded from caudate.

Pretreatment with PCPA also failed to inhibit the amphetamine-induced increase in P2 in both caudate and globus pallidus Dialysis studies have confirmed that amphetamine enhances the release of UA in caudate but has no detectable effect on 5HIAA [19]. Therefore, the failure of PCPA to inhibit the amphetamine-induced increase in P2 in caudate is consistent with previous research. Note that microinfusion of uricase does eliminate the amphetamine-induced increase in P2 in caudate [15]

Even though globus pallidus contains far more 5HT and

5HIAA than caudate, PCPA had little effect on the amphetamine-induced increase in P2 in globus pallidus. Amphetamine does produce a strong effect in the globus pallidus, but this effect depends upon DA with little or no contribution from 5HT neurons [1]. Thus the hypothesis that the amphetamine-induced increase in P2 reflects UA rather than indoles is consistent with the available literature

One might argue that PCPA failed to have the expected effects on P2 because 5HT depletion did not occur for some reason. However, several observations suggest that PCPA was, in fact, effective. First, the animals' behavior was dramatically affected on the day of the PCPA injection. Second, animals pretreated with PCPA were much more sensitive to amphetamine than controls (see below) and many were very difficult to handle Finally, PCPA has been shown to be a very reliable and effective tool for depleting 5HT.

Amphetamine produced approximately the same increase in AA in both caudate and globus pallidus, even though anterior caudate contains far more dopamine than globus pallidus On the other hand, the PCPA-induced potentiation of AA release occurred only in caudate Thus changes in extracellular AA levels are not entirely nonspecific. Pretreatment with PCPA enhances some of the behavioral effects of amphetamine [11]. In this context the PCPA-induced potentiation of AA release in caudate becomes very interesting Changes in AA levels in caudate appear to mirror changes in behavior in at least this instance. (Although previous data indicate that P1 monitors AA [2,14], there is a possibility that dopamine may contribute to the PCPA-induced potentiation of P1 in caudate)

In conclusion, neither PCPA nor the location of the electrodes had any effect on P2 In each case P2 recorded from caudate responded similarly to P2 recorded from globus pallidus Since P2 recorded from caudate has already been shown to reflect UA rather than 5HIAA or 5HT, these data suggest that P2 recorded from globus pallidus also monitors UA.

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