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Effects of diazepam on regional brain homovanillic acid following phencyclidine or Δ^9-tetrahydrocannabinol

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Some drugs which can produce or exacerbate psychotic states in humans preferentially activate dopaminergic neuronal pathways in rat brain. Under certain conditions, phencyclidine (PCP) and Δ^9-tetrahydrocannabinol (THC) increase homovanillic acid (HVA) in limbic and cortical regions at doses that do not affect caudate HVA [1, 2]. Mild footshock stress in rats produces a similar selective activation of dopaminergic pathways, an effect which can be antagonized by diazepam [3–6]. In this report, we describe the effects of diazepam upon the regional increase in HVA produced by PCP and THC.

Materials and methods

Male Sprague–Dawley rats (Charles River, 250 mg) were used. Rats were injected with diazepam (2 or 5 mg/kg intraperitoneally) at 90 min and either PCP (5 mg/kg) or THC (20 mg/kg) at 60 min prior to killing the animals. Control animals received saline or diazepam alone. Brain samples for prefrontal cortex, olfactory tubercle, and caudate were obtained as previously described and frozen at −60° until assayed for HVA by gas chromatography–mass spectrometry using deuterated internal standards [7]. PCP and THC were provided through the courtesy of the Research Technology Branch, Division of Research, NIDA. PCP was dissolved in aequeous solution. THC was prepared for injection using 0.1 ml Tween 80, dried under nitrogen, and resuspended in saline. Injectable diazepam was purchased from commercial sources. The effects of diazepam upon THC- and PCP-induced regional increases in HVA were assessed with a one-way ANOVA and the Bonferonni *post hoc* test [8].

Results and Discussion

PCP produced a significant increase in HVA in olfactory tubercle and prefrontal cortex but not in caudate (Table 1). When animals were pretreated with diazepam, the effect of PCP in olfactory tubercle and prefrontal cortex was partially antagonized. Thus, following PCP, HVA in the olfactory tubercle and prefrontal cortex of diazepam-treated animals was significantly lower than the HVA in these regions from animals who received PCP alone but still significantly higher than in the saline-treated or diazepam-treated animals. Diazepam alone decreased HVA in the

Table 1. Effect of PCP upon regional brain HVA following diazepam pretreatment

	HVA (ng/g)		
	Caudate	Olfactory tubercle	Prefrontal cortex
Saline	982 ± 35	425 ± 29	65 ± 4
Diazepam	820 ± 42*	351 ± 12	56 ± 3
PCP	914 ± 49	643 ± 43†	129 ± 7†
Diazepam + PCP	814 ± 35*	507 ± 34‡	83 ± 6§

Each value is the mean ± SE of seven to eight animals. Diazepam (2 mg/kg, i.p.) was administered at 90 min and PCP (5 mg/kg, i.p.) at 60 min prior to killing the animals.

* $P < 0.01$ compared to saline. Not significantly different from PCP.

† $P < 0.01$ compared to saline or diazepam.

‡ $P < 0.01$ compared to PCP, $P < 0.05$ compared to saline and diazepam.

§ $P < 0.001$ compared to PCP, $P < 0.05$ compared to saline and diazepam.

caudate, and this effect was not altered by PCP. Table 2 shows the results of the diazepam–THC study. THC significantly increased HVA in olfactory tubercle and prefrontal cortex. Pretreatment with diazepam had no effect upon the ability of THC to increase HVA in these two regions. Diazepam at 5 mg/kg also failed to alter the THC-induced regional increase in HVA (data not shown).

THC increases HVA in all three brain regions at higher doses; however, at lower doses THC preferentially increases HVA in olfactory tubercle and prefrontal cortex [2]. PCP actually produces a decrease in caudate HVA and an increase in HVA in olfactory tubercle and prefrontal cortex [1, 9]. Thus, both compounds can increase HVA preferentially in olfactory tubercle and prefrontal cortex. This pattern is similar to the increase in regional HVA produced by footshock stress [10]. Our data show that diazepam, which antagonizes the HVA increase produced by footshock, also modified the HVA increase following PCP but not that following THC at least at the two doses used in the present study. It thus appears that there are similarities as well as differences between the drug and stress procedures which increase HVA preferentially in rat forebrain. We have shown recently that PCP and footshock stress may increase regional HVA by somewhat distinct mechanisms [11]. We came to this conclusion based upon the additive effects on cortical HVA that we observed when the two were combined and upon differential changes in substance P and substance K in the ventral tegmental area when either footshock or PCP was administered. We found subsequently that THC had no effect upon either substance P or substance K in the ventral tegmentum (unpublished data). With regard to diazepam treatment, the current experiments indicate that it is partially effective in reversing the increase in cortical and limbic HVA produced by PCP but not that produced by THC. Therefore, some similarity is suggested in the mechanisms whereby footshock and PCP increase forebrain HVA. The locus of action for diazepam in antagonizing the effects of footshock and PCP may be through the inhibitory effect of γ-aminobutyric acid (GABA) receptors acting upon dopaminergic neurons in the ventral tegmental area. PCP may produce a less selective stimulation of dopaminergic neurons in this region by a more potent effect upon tachykinin release [11].

It is known that PCP binds to specific receptors in brain [12, 13]. Although the precise relationship between PCP receptors and the dopamine system has not yet been determined, one lesion study suggests that PCP binding sites may be located on mesolimbic dopaminergic terminals [14].

The mechanism of the effect of THC upon the dopamine system has not been investigated extensively. There is some evidence that the synthesis of dopamine from tyrosine is enhanced by cannabis, suggesting an increase in tyrosine hydroxylase activity [15]. Such a metabolic effect might be less subject to modification by diazepam.

It thus appears that an increase in dopamine metabolism in rat forebrain may occur as the result of different mechanisms. If the same holds for primates and humans, there may be analogies in clinical states characterized by pathological arousal where apparently similar conditions may result from somewhat different neural mechanisms and show varied treatment responses.

Table 2. Effect of THC upon brain HVA following diazepam pretreatment

	HVA (ng/g)		
	Caudate	Olfactory tubercle	Prefrontal cortex
Saline	906 ± 41	405 ± 30	53 ± 6
Diazepam	1015 ± 24	378 ± 20	44 ± 4
THC	1128 ± 70	713 ± 29*	82 ± 6*
Diazepam + THC	1007 ± 58	650 ± 30†	80 ± 7†

Each value is the mean ± SE of four to seven animals. Diazepam (2 mg/kg, i.p.) was administered at 90 min and THC (20 mg/kg, i.p.) at 60 min prior to killing the animals.

* $P < 0.01$ compared to saline or diazepam.

† $P < 0.01$ compared to saline or diazepam. Not significantly different from THC.

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Reversible and irreversible inhibition of hepatic mitochondrial respiration by acetaminophen and its toxic metabolite, *N*-acetyl-*p*-benzoquinoneimine (NAPQI)

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Acetaminophen (AA) is an analgesic and antipyretic drug that has become a popular alternative to aspirin as a readily available over-the-counter pain reliever. While considered to be safe when taken in therapeutic doses, it has become evident that the drug is capable of causing severe centrilobular liver damage when taken in large quantities [1, 2]. The hepatotoxic action of AA has been suggested to be the result of activation of the drug by hepatic mixed-function oxidase (MFO) to its toxic metabolite *N*-acetyl-*p*-benzoquinoneimine (NAPQI) [3]. High doses of AA have been found to saturate the glucuronide and sulfate conjugating systems in the liver which allows more of the drug to be metabolized by MFO to NAPQI [4, 5]. In the presence of sufficient levels of glutathione (GSH), the NAPQI formed in the hepatocyte is thought to be detoxified through conjugation with GSH or by reduction of the NAPQI back to AA by GSH [6]. The eventual depletion of GSH from the cell, resulting from the formation and excretion of the GSH conjugate, leaves essential sulfhydryl-containing enzymes within the cell vulnerable to NAPQI. NAPQI has the potential to react with sulfhydryl groups through the formation of covalent adducts [7–10] or through sulfhydryl

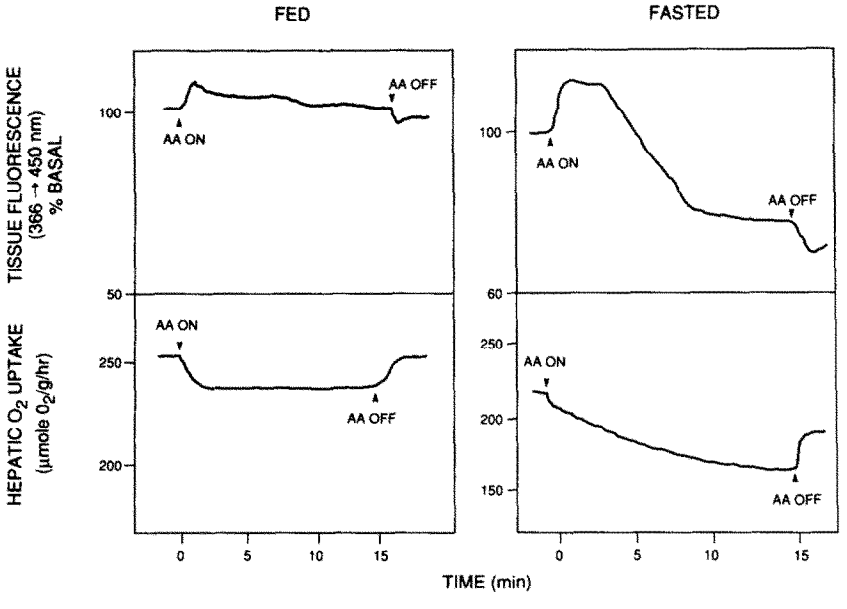

Fig. 1. Left panel: Effect of 5 mM AA perfused through a control liver. Right panel: Effect of 5 mM AA perfused through an isolated liver from a 48-hr fasted rat. Krebs–Henseleit bicarbonate buffer containing 5 mM AA, pH 7.4, was perfused through the isolated rat liver for 15 min. The resulting alterations in hepatic oxygen uptake and tissue fluorescence were determined as described in Materials and Methods. Data presented are representative of three liver perfusions for each treatment.