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EFFECTS OF PENTYLENETETRAZOL AND TRIMETHADIONE ON FELINE BRAIN MONOAMINE METABOLISM

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Abstract—The effects of pentylenetetrazol on behavioral excitation and brain monoamine metabolism were compared by monitoring the EEG and assaying feline cerebrospinal fluid (CSF) for monoamine metabolites. After a non-convulsant dose of pentylenetetrazol, neither the concentrations of the 5-hydroxytryptamine (5-HT) metabolite, 5-hydroxyindoleacetic acid (5-HIAA), nor the dopamine (DA) metabolite, homovanillic acid (HVA), were altered in CSF if the rectal temperature of the cat was maintained. After a convulsant dose there was an increase in 5-HIAA and HVA levels. The norepinephrine (NE) metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), was also increased, but returned to control within 3 hr, while 5-HIAA and HVA levels were elevated for 24 hr. Trimethadione produced a transient decrease in HVA levels. When the convulsions, but not EEG excitation, are prevented by trimethadione pretreatment, brain monoamine metabolism is increased. Plasma tryptophan levels decreased after convulsant doses of pentylenetetrazol. Pentylenetetrazol was not detectable in plasma or CSF 24 hr after injection, while CSF 5-HIAA and HVA levels were still increased. These data show that pentylenetetrazol directly increases brain NE, DA and 5-HT metabolism while causing EEG excitatory changes, an effect which may precede convulsions.

We previously reported that the analeptic drug pentylenetetrazol increased feline brain 5-hydroxytryptamine (5-HT) metabolism after nonconvulsant doses and increased both brain 5-HT and dopamine (DA) metabolism after convulsant doses [1]. The increase in 5-HT metabolism followed a decrease in rectal temperature. A decline in body temperature increases the release of 5-HT into push–pull perfusates in monkeys [2] and increases the concentration in feline cerebrospinal fluid (CSF) of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) [3]. We concluded that the increased concentration of 5-HIAA in CSF could be, in part, due to the decline of body temperature [1]. However, since the increased 5-HIAA concentration in CSF lasted for 24 hr, and the decrease in body temperature lasted for 2–4 hr, additional factors may be involved in the increased 5-HT metabolism. In rats, convulsions induced by pentylenetetrazol increase brain levels of 5-HT for 10 min to 24 hr [4, 5] and increase norepinephrine (NE) levels for 1 hr followed by decline to pre-drug values [5]. However, the half-life for plasma disappearance of pentylenetetrazol in rats is less than 4 hr [6], which indicates that only trace amounts of drug are present 24 hr after administration, while 5-HT levels are still increased. It appears that changes occur after administration of pentylenetetrazol that persist after the drug is removed from the body.

The following experiments were designed to clarify relationships between brain monoamine metabolism and the behavioral and temperature responses of cats to pentylenetetrazol. To determine the relative contributions of pentylenetetrazol and the decline in body temperature to the increase in 5-HT metabolism, pentylenetetrazol was administered to cats, and the body temperature maintained by elevating the ambient temperature. To determine the relationship between drug level and effect, we compared the time course of plasma and CSF pentylenetetrazol disappearance with the time course for alterations of brain monoamine metabolism. Since pentylenetetrazol may alter peripheral tryptophan (TRYP) metabolism, which could affect brain 5-HT metabolism [7], total TRYP levels in plasma were measured after administration of pentylenetetrazol in order to compare the time course of plasma concentrations with the time course for alterations of brain 5-HT metabolism. Finally, trimethadione, an anticonvulsant drug that antagonizes the effects of pentylenetetrazol [8, 9], was administered alone or as a pretreatment to pentylenetetrazol, and the time courses for brain monoamine metabolite concentrations in feline CSF were compared to the behavioral responses of the drug treatments. In all the above experiments an increase in concentration of a monoamine metabolite in CSF was interpreted as an increase in the metabolism of functional amines in brain tissue [10–12].

METHODS

Cats (2.5 to 4.5 kg) were maintained in separate cages with a 12-hr light–dark cycle. A chronic indwelling cannula was implanted in the cisterna magna for serial sampling of CSF [13]. To obtain serial samples of blood, a chronic catheter in the right external jugular vein was placed 1–2 cm from

the heart and anchored in dental cement on top of the skull. Animals were allowed a week to recover from surgery before any experimentation.

The metabolites of 5-HT and DA, 5-HIAA and homovanillic acid (HVA), respectively, were initially determined in 1.0 ml CSF by combining extraction and fluorometric methods described by others [14, 15]. In later experiments, analyses for HVA, and 3-methoxy-4-hydroxy-phenylethyleneglycol (MHPG), an NE metabolite, were performed using a gas chromatographic electron capture detection method. This method combined extraction and fluorinated derivatizing procedures described by others [16–18]. 5-HIAA was quantitated by fluorometry [14, 15]. Total plasma TRYP concentrations were determined in duplicate 30-μl aliquots using a modification [19] of the fluorometric method of Denkla and Dewey [20]. Pentylenetetrazol was quantitated in plasma and CSF using the method of Vohland and Koransky [6]. A rotary flash evaporator was used to evaporate the $CHCl_3$ extract, which resulted in more consistent recoveries.

Our experimental design allows us to use each cat as its own control. The monoamine metabolite concentrations in the first sample of CSF of the day are taken as the animal's baseline and set to a value of 100. The samples following are compared with it as a percentage change. The response to a solution containing drug is always compared to the response of a solution not containing drug in the same animal. Samples are taken at the same times of day both on the vehicle control day and the experimental day. Thus, between cats, variations in monoamine metabolite levels are reduced. The paired Student's *t*-test [21] is used for determining significant differences between pairs of values from each cat for each group of cats given the same dose of drug.

RESULTS

Effects of pentylenetetrazol and body temperature on brain monoamine metabolism. The effect of pentylenetetrazol to reduce body temperature may account for the increased levels of 5-HIAA in CSF [1]. To test this possibility, cats were given 20 or 40 mg/kg of pentylenetetrazol i.p. and their rectal temperatures were maintained by increasing ambient temperature using an insulated box with a hot air blower inside. After a 20 mg/kg dose, exposure to 35° air temperature for 30 min was sufficient to maintain the rectal temperature of animals. However, controlling the animals' temperature after 40 mg/kg proved to be difficult. The animals pant after the higher dose of pentylenetetrazol for at least 1 hr, and an ambient temperature of 39–41° for 2 hr was necessary to maintain their temperature within control values after convulsions. Exposure of control animals to these conditions caused a temperature increase of 0.5 to 1.0° and some intermittent panting. Because these extreme conditions add more variables to the experimental design, pentylenetetrazol-induced convulsions with the temperature controlled was compared to drug-induced convulsions without an increased ambient temperature.

Figure 1 shows that maintaining the rectal temperature constant after a nonconvulsant dose of 20 mg/kg of pentylenetetrazol i.p. prevented the increase in 5-HIAA concentrations seen previously with this dose [1]. An analysis of variance on the samples withdrawn during the control period (saline injection) showed that 5-HIAA and HVA did not change significantly from the values of 148.0 and 137.1 ng/ml respectively (see legend to Fig. 1). HVA levels were unaffected, as in the previous experiments. Figure 2 shows the effects of 40 mg/kg of pentylenetetrazol with and without the temperature controlled, on 5-HIAA concentrations in CSF. Although the rectal temperature was maintained within 1.0° of pre-drug values (compared to an average decrease of 3.1° in rectal temperature without the increased ambient temperature), the 5-HIAA concentrations were increased similarly to the same dose without the temperature controlled. These data indicate that pentylenetetrazol-induced convulsions can increase brain 5-HT metabolism, but after nonconvulsant doses, the increased 5-HT metabolism follows the decrease in rectal temperature.

Time course of pentylenetetrazol disappearance. Pentylenetetrazol was measured in plasma and CSF in order to compare the time course of drug in the body with the time course of changes of brain monoamine metabolism, because pentylenetetrazol produced a change in brain 5-HT and DA metabolism persisting up to 24 hr. The time course of pentylenetetrazol disappearance from plasma and CSF after injection of 20 mg/kg of drug is shown in Fig. 3. Values are corrected for the water content of the two fluids where plasma is 91% water and CSF is 99% [22]. This figure shows that pentylenetetrazol is

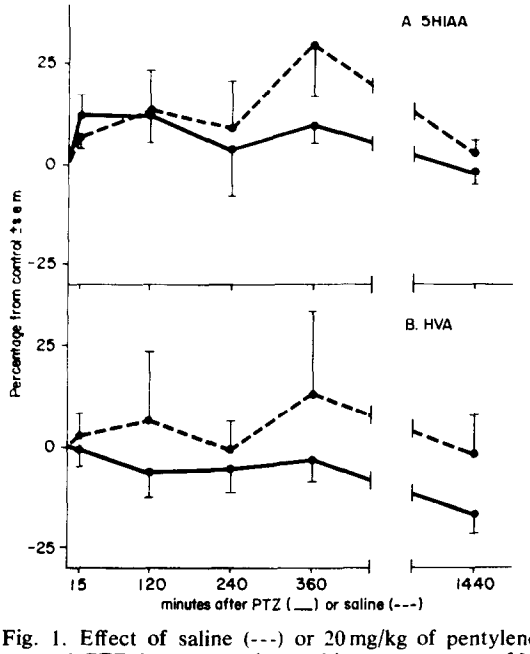

Fig. 1. Effect of saline (---) or 20 mg/kg of pentylenetetrazol (PTZ) i.p. (——) and an ambient temperature of 35° for 30 min after injection on (A) 5-HIAA and (B) HVA concentrations in CSF. Each point represents the average percentage change for three cats ± S.E.M. Control values (ng/ml) for 5-HIAA and HVA before saline were 148.0 ± 21.0 and 137.1 ± 37.0 and before PTZ were 151.7 ± 20.8 and 136.5 ± 38.3 respectively.

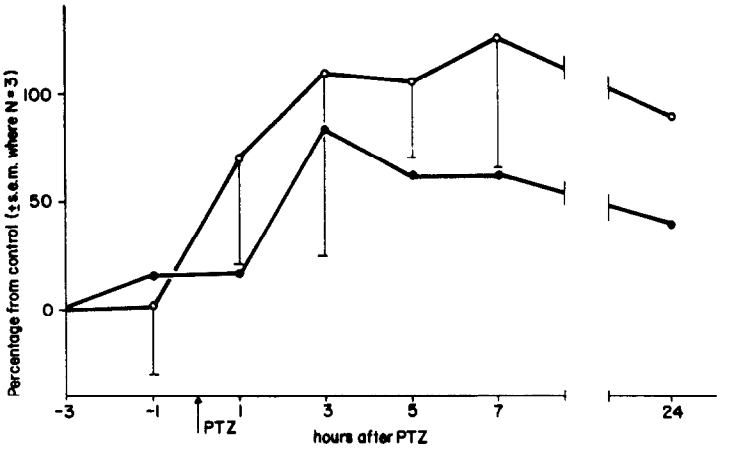

Fig. 2. Effect of 40 mg/kg of PTZ i.p. injected at the arrow with (●) (control value 108.1 ± 48.1 ng/ml) or without (○) (control value 89.4 ± 46.3 ng/ml) an ambient temperature of 39–41° for 2 hr after injection on 5-HIAA levels in CSF. Each point represents the average percentage change for three cats ± S.E.M. for the experiments without the controlled temperature and the percentage change of two cats for the experiments with the controlled temperature.

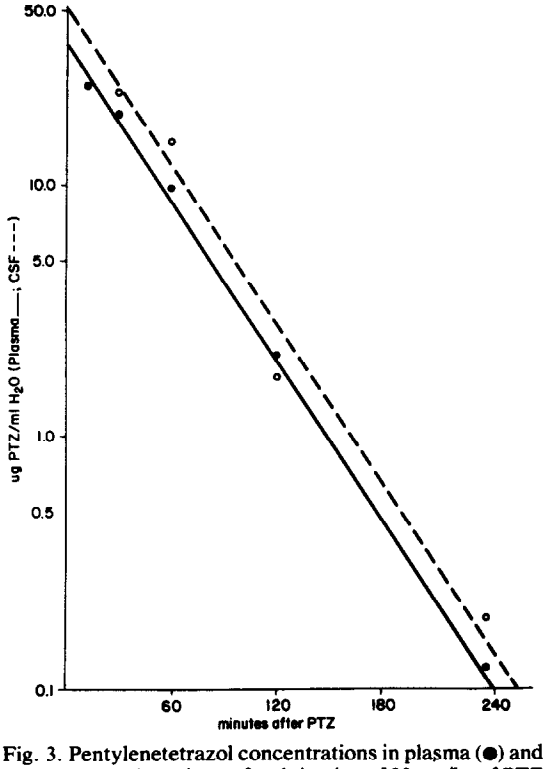

Fig. 3. Pentylenetetrazol concentrations in plasma (●) and CSF (○) at various times after injection of 20 mg/kg of PTZ i.p. Fluids were sampled simultaneously from the same cat, and values were corrected for water content (plasma, 91 per cent and CSF, 99 per cent).

rapidly absorbed after intraperitoneal injection, that the elimination of pentylenetetrazol occurs at the same rate from these two fluid compartments and that pentylenetetrazol rapidly equilibrates between plasma and CSF.

Table 1 reports the estimated half-life and zero-time concentrations of pentylenetetrazol in plasma and CSF. The half-life of pentylenetetrazol in plasma is significantly longer after 40 mg/kg than after 20 mg/kg of drug. Analysis of variance indicated that the slope of the plasma elimination phase (0.00462 min^{-1}) after the higher dose is significantly different from the slope (0.01348 min^{-1}) after 20 mg/kg. However, the slope of 0.01348 min^{-1} for plasma elimination is not different from the slope of 0.01833 min^{-1} for CSF elimination after 20 mg/kg of pentylenetetrazol. Calculations showed that the drug concentration in these fluids after a 40 mg/kg dose of pentylenetetrazol, allowing six half-lives to elapse, would be at or below the detection limits of the assay procedure. In harmony with this, pentylenetetrazol was undetectable in plasma or CSF sampled 24 hr after injection of either dose of drug. These data indicate that the elimination of pentylenetetrazol is dose dependent and that the drug is not present in the circulation to exert an effect 24 hr after injection.

Effects of pentylenetetrazol on plasma tryptophan. The prolonged effects of pentylenetetrazol on brain 5-HT metabolism may reflect alterations in the peripheral metabolism of tryptophan [7, 23]. To determine whether a peripheral action of pentylenetetrazol causes the change in brain 5-HT metabolism, pentylenetetrazol was given to cats and TRYP was determined in serial samples of plasma. Figure 4 shows these data. The nonconvulsant dose of pentylenetetrazol did not alter TRYP concentrations in plasma. However, the convulsant dose of 40 mg/kg caused a decrease in total plasma TRYP. This result is the opposite of the change in CSF 5-HIAA

Table 1. Estimated half-life and initial concentration (T = 0) of PTZ in CSF and plasma* ·

Dose of PTZ (i.p.)	Half-life (min)	Concn of PTZ at T = 0 (μg/ml)
20 mg/kg	37.8 ± 7.4†	48.7 ± 11.5†
	51.4 ± 13.7‡	28.5 ± 6.6‡
40 mg/kg	149.8 ± 22.0‡	59.2 ± 23.2‡

* Results are from three to six cats and are expressed as mean ± S.D.
† In CSF.
‡ In plasma.

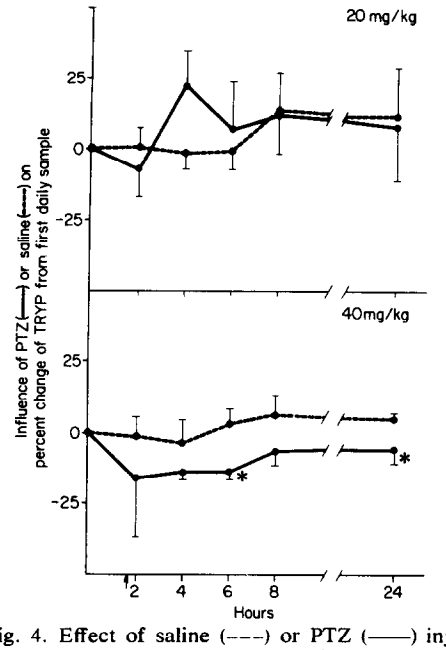

Fig. 4. Effect of saline (---) or PTZ (——) injected at arrow on plasma concentrations of tryptophan (TRYP). Each point represents the average percentage change of four to five cats ± S.E.M. Control values (μg/ml) were 7.7 ± 1.1 and 8.3 ± 1.3 before saline or 20 mg/kg PTZ i.p. and 10.8 ± 0.7 and 11.3 ± 0.9 before saline or 40 mg/kg PTZ i.p. respectively.

concentrations reported above. Therefore, the 24-hr increase in CSF 5-HIAA levels does not follow an increase in plasma TRYP levels, but may be a direct effect of pentylenetetrazol on 5-HT neurons.

Effects of trimethadione. Trimethadione competitively inhibits convulsions produced by pentylenetetrazol, possibly at the receptor level [8]. To determine whether trimethadione alters brain monoamine metabolism, it was given to cats and the CSF was periodically sampled for assay of monoamine metabolites. A dose of 200 mg/kg i.p. produced sedation and ataxia for at least 2 hr. The behavioural sedation was accompanied by a synchronized EEG which could be interrupted for only a few min by a sudden loud noise. Thirty min after this dose, HVA levels in CSF were decreased relative to the vehicle controls (vehicle was 7.0 ml of 20% ethanol, v/v, which is an average dose of 0.36 g/kg of ethanol). Note in Fig. 5 that the ethanol has increased the HVA levels (compare with the effect of saline on HVA levels shown in Fig. 1B) and that the HVA concentrations were within the limits of the vehicle control for the rest of the sampling period. Trimethadione did not alter 5-HIAA levels (Fig. 5). Significant protection against pentylenetetrazol convulsions provided by trimethadione has been reported to last up to 6 hr [24]. Our data suggest that the anticonvulsant property of trimethadione is not mediated by a change in brain 5-HT or DA metabolism.

When 40 mg/kg of pentylenetetrazol was injected 90 min after trimethadione, the sedation and ataxia were rapidly reversed and the animals became alert and slightly agitated. Neither myoclonus nor convulsions occurred, but EEG seizures or some agitation was observed in these animals. Figure 6A shows the effects of 40 mg/kg of pentylenetetrazol on CSF levels of 5-HIAA, HVA and MHPG, and Fig. 6B shows the effect of pretreating the animals with trimethadione. Pentylenetetrazol elevated 5-HIAA and HVA levels, whether or not trimethadione was injected. The duration of the increase of MHPG levels was diminished by the trimethadione pretreatment and 5-HIAA levels were within control values by 24 hr. These data suggest that pentylenetetrazol can directly increase brain monoamine metabolism in the absence of convulsions while still causing EEG seizures.

DISCUSSION

Diaz [25] reported that a sub-convulsant dose of pentylenetetrazol in rats decreases 5-HIAA and increases 5-HT levels in whole brain during the first hr after injection. This result is opposite to what we reported above. This discrepancy in results may be explained by differences in the period of observation since Diaz examined 5-HT metabolism from 5 to 60 min while the increase in CSF 5-HIAA levels in cats occurred 240 min after injection of drug. Alternatively, there may be a difference between species. Preventing the body temperature decline (Fig. 1) prevented the increase in 5-HT metabolism, indicating that the increased 5-HT metabolism follows the effect of pentylenetetrazol to decrease body temperature. The rat appears to have a different

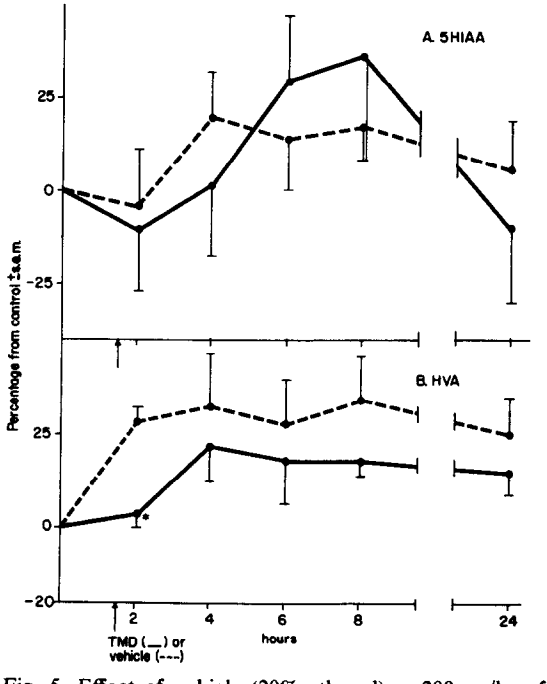

Fig. 5. Effect of vehicle (20% ethanol) or 200 mg/kg of trimethadione (TMD) i.p. on (A) 5-HIAA and (B) HVA levels in CSF. Each point represents the average percentage change of five to six cats ± S.E.M. Control values (ng/ml) for 5-HIAA and HVA before vehicle were 93.1 ± 17.7 and 62.7 ± 5.2 and before TMD were 110.6 ± 15.0 and 76.8 ± 4.4 respectively. Significant differences from vehicle control samples have been labeled with an asterisk where P is at least less than 0.05.

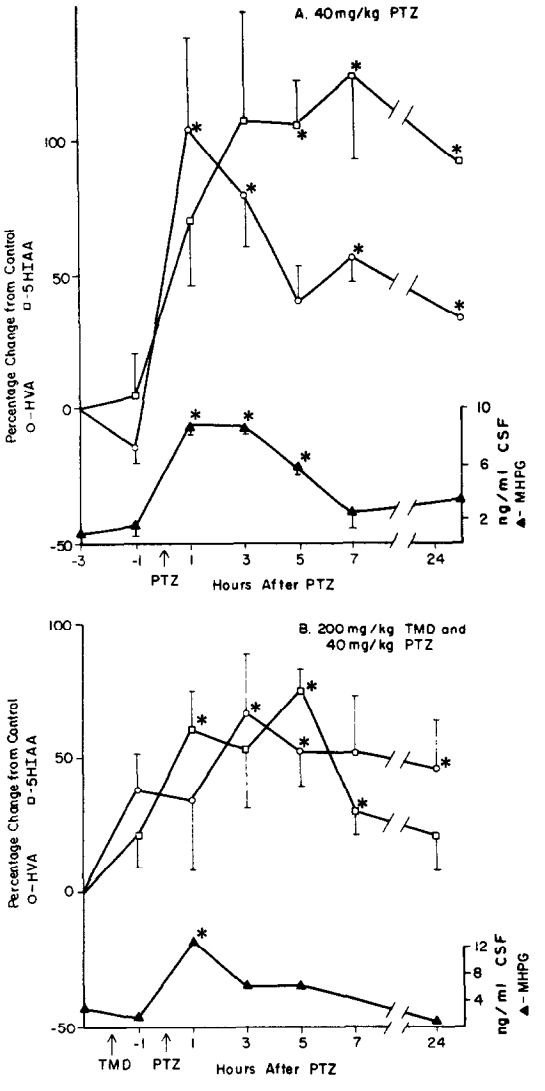

Fig. 6. Effect of (A) 40 mg/kg of PTZ i.p. or (B) 200 mg/kg of TMD i.p. and 40 mg/kg of PTZ i.p. on 5-HIAA, HVA and MHPG levels in CSF. Each point represents the average percentage change of three cats ± S.E.M. for 5-HIAA and HVA. Control values (ng/ml) for 5-HIAA and HVA in (A) were 89.4 ± 46.3 and 63.0 ± 11.7 and in (B) were 74.0 ± 16.4 and 61.9 ± 8.8 respectively. Because of the low control values of MHPG (▲) obtained in our cats, these data are expressed as ng/ml of CSF. Significant differences from vehicle control samples have been labeled with an asterisk where P is at least less than 0.05.

relationship between body temperature and forebrain 5-HT metabolism, since increased body temperature has been reported to increase 5-HT metabolism [26]. The effect of pentylenetetrazol on the body temperature of rats was not examined by Diaz [25], making comparisons with our results difficult. Prolonged effects of convulsant doses of pentylenetetrazol for up to 24 hr on brain 5-HT levels and for 1 hr on NE levels have previously been noted to occur in rats [5]. Electroconvulsive shock has been reported to produce prolonged increases in 5-HT levels in rat brain [4, 5] and increased 5-HT metabolism 1 and 3 hr after administration of electroconvulsive shock [27, 28]. It appears that serotonergic systems recover less rapidly than noradrenergic systems from single convulsions.

When a convulsant dose of pentylenetetrazol is administered after trimethadione pretreatment, the behavioral effects are partially inhibited (i.e. no convulsions occur, but agitation or EEG seizures appear) while brain 5-HT metabolism and DA metabolism increase. It appears that an increase in DA and 5-HT metabolism with an increase in EEG activity is a result of pentylenetetrazol acting directly to increase neuronal excitability. It is speculative to relate the agitation and EEG seizures caused by pentylenetetrazol to the increase in amine metabolism.

The effects of pentylenetetrazol on plasma total TRYP concentrations were examined in search of an explanation for the persistent increase in 5-HT metabolism after pentylenetetrazol disappears from the body. After non-convulsant doses of pentylenetetrazol, plasma TRYP levels were unaffected, while CSF 5-HIAA levels increased. We demonstrated that the increased 5-HT metabolism after nonconvulsant doses is secondary to the decline in body temperature (Fig. 1). On the other hand, after a convulsant dose of pentylenetetrazol which increases 5-HT metabolism, even when the fall in body temperature is prevented (Fig. 2), total TRYP levels decrease for up to 24 hr (Fig. 4). However, these results are only suggestive because the levels of brain TRYP may depend on a variety of factors in addition to total TRYP, including: levels of plasma-free TRYP, neutral aromatic amino acids that compete for uptake into the brain, and insulin [7, 29]. In addition, our results show a different pattern of monoamine metabolism than that induced by immobilization stress which decreases both plasma TRYP and brain DA metabolism in rats [20, 30, 31] and increases 5-HT metabolism only after 4–6 hr of continuous immobilization [20, 30, 32]. Thus, our observations support the contention that pentylenetetrazol increases brain 5-HT metabolism by a mechanism other than the stress of convulsions.

Pentylenetetrazol is rapidly absorbed and distributed when administered either orally [33] or parenterally [6, 33]. The drug exhibits a rapid distribution phase [33] such that plasma levels reach a maximum and fall rapidly within 30 min. Kirsten and Schoener [9] report that the neuronal responses to small intravenous doses of pentylenetetrazol are brief, which is in harmony with the pharmacokinetics of the drug. However, pentylenetetrazol alters brain monoamine metabolism for a prolonged period. Plasma pentylenetetrazol levels are below detection limits 24 hr after the administration of nonconvulsant or convulsant doses (Fig. 3 and Table 1) while changes in monoamine metabolism persist. Vohland and Koransky [6] report that the disappearance of pentylenetetrazol from brain tissue parallels the time course of drug levels in plasma, indicating that the drug is not sequestered in brain tissue to produce its effects 24 hr after injection and that plasma levels reflect drug concentration at its site of action (i.e. brain tissue). In

addition, 70–95 per cent of a dose of pentylenetetrazol appears in the urine of rats within 24 hr, largely as metabolites [6, 34]. Therefore, the prolonged effects of pentylenetetrazol on brain monoamine metabolism are a result of some mechanism other than continued presence of drug.

We have interpreted increased CSF metabolite concentrations as an increase in the metabolism of the parent amine. An alternative interpretation is that a decrease in the rate of metabolite efflux occurs. This argument does not apply to MHPG, which is a non-ionized and freely diffusable molecule [35]. If the efflux of 5-HIAA and HVA were inhibited, then changes in their CSF concentrations should qualitatively follow the same pattern since these two acids compete for transport by the same probenecid-sensitive site [11]. However, 5-HIAA and HVA levels follow different patterns, as shown in Figs. 5 and 6. In addition, neither pentylenetetrazol nor its major metabolites (6-hydroxy and 8-hydroxypentylenetetrazol [36]) are acids and would not be expected to inhibit organic acid transport. Thus, the increases seen after pentylenetetrazol probably represent increases in the metabolism of the parent amine.

Our results suggest several relationships between brain monoamine metabolism and the behavioral responses to pentylenetetrazol. First, after non-convulsant doses of drug, brain 5-HT metabolism increases correlate with the decline in rectal temperature. This result is in harmony with earlier reports that the serotonergic neuronal system acts to maintain body temperature in cats and primates [2, 3]. Second, trimethadione does not alter brain monoamine metabolism during its anticonvulsant action. Finally, behavioral responses to pentylenetetrazol (agitation and EEG seizures) can be temporally related to changes in brain amine metabolism. The prolonged biochemical responses are independent of circulating drug and may represent persistent changes in neuronal activity.

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