Br. J. Pharmac. (1975), 53, 299-304

THE FAILURE OF *p*-CHLOROPHENYLALANINE TO AFFECT VOLUNTARY ALCOHOL CONSUMPTION IN RATS

R.B. HOLMAN, VALERIE HOYLAND & ELIZABETH E. SHILLITO

Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

1 The effects of *p*-chlorophenylalanine (PCPA) administered orally and intraperitoneally on rat brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) content were compared. The depletion of brain 5-HT and 5-HIAA following PCPA (316 mg/kg) injected intraperitoneally every third day was not significantly different from that following the administration of PCPA (316 mg/kg) by stomach tube on eight consecutive days.

2 Rats tested for alcohol preference before, during and after treatment with PCPA on two intraperitoneal dose regimens (either 316 mg/kg then 100 mg/kg four days later or 316 mg/kg three times at intervals of three days) showed no reduction in voluntary alcohol consumption.

3 The results indicated that depletion of brain 5-HT and 5-HIAA is not responsible for the reduction of voluntary alcohol intake which has been reported to follow chronic oral administration of PCPA to the rat (Myers & Veale, 1968). The possibility of a learned aversion to alcohol due to an association with PCPA administration is discussed.

Introduction

Several reports have suggested a relation between cerebral 5-hydroxytryptamine (5-HT) and voluntary alcohol consumption in rats. Ahtee & Eriksson (1972) found that rats, genetically selected for their alcohol preference, had higher brain concentrations of 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) than rats that preferred to drink water. When rats were treated with *p*-chlorophenylalanine (PCPA) to lower cerebral 5-HT (Koe & Weissman, 1966) and tested for consumption of tap water and alcohol their alcohol intake was reduced after drug treatment (Myers & Veale, 1968; Cicero & Hill, 1970; Nachman, Lester & Le Magnen, 1970; Veale & Myers, 1970). Myers & Veale (1968) found that this effect occurred not only during the drug administration but was maintained up to two months after PCPA treatment ceased, when presumably the brain 5-HT concentration had returned to normal. PCPA also decreased alcohol consumption by the rat under conditions in which intake normally increased (Veale & Myers, 1970; Myers, Evans & Yaksh, 1972; Myers, 1972). As well as PCPA, *p*-chloroamphetamine depleted brain 5-HT concentrations and reduced the frequency of selection of 3%, 5% or 10% alcohol solutions when offered as a choice with tap water (Frey, Magnussen & Nielsen, 1970). This decreased intake also lasted after the drug treatment had ended.

In these tests for consumption of increasing concentrations of alcohol, PCPA has been administered daily for ten days or more by stomach tube in doses of 200 mg/kg to 300 mg/kg. Only Frey *et al.* (1970) measured brain 5-HT content at the end of oral administration of PCPA (300 mg/kg) during alcohol preference tests and found the concentration was reduced to 20% of the normal value. To determine if changes in cerebral 5-HT metabolism are crucial in regulation of voluntary alcohol consumption, the time course and magnitude of 5-HT depletion following PCPA administration must be determined over the extended time periods required for alcohol preference testing. Koe & Weissman (1966) reported that four or five daily oral doses of PCPA (100 mg/kg) or a single intraperitoneal dose of PCPA (316 mg/kg) resulted in the depletion of brain 5-HT to 7-12% of the control value after three to four days. Shillito (1970) has found that reduced concentrations of brain 5-HT can be maintained with intraperitoneal injections of PCPA at three day intervals. The following experiments were carried out to examine (1) the effects of oral and intraperitoneal administration of PCPA on brain 5-HT and 5-HIAA content with

and without alcohol preference testing; and (2) the effect of intraperitoneal administration of PCPA on voluntary alcohol consumption.

Methods

The animals used were male albino rats of the Wistar strain supplied by A. Tuck & Son (Laboratory Animal Breeding Station, Rayleigh, Essex); they weighed about 150 g at the start of each experiment. The rats were housed in individual cages with food and water *ad lib.* They were kept under reversed daylight conditions in which a red light was on from 10 h 00 min to 22 h 00 min and white light for the remaining 12 hours. The animals were adapted to the reversed daylight conditions for at least a week before any experiments were begun. All routine maintenance, fluid intake and weight readings, PCPA injections and decapitations for brain 5-HT and 5-HIAA estimations were carried out between 09 h 30 min and 10 h 00 minutes.

Drug administration

PCPA was administered orally by gastric tube in daily doses of 316 mg/kg. The PCPA (31.6 mg/ml) was suspended by agitation in 20 ml of 0.9% w/v NaCl solution (saline) acidified to pH 1.0 with 0.1M HCl and then the pH was raised to 4.5 with 0.1M NaOH as described by Myers & Veale (1968). When intraperitoneal injections of PCPA (316 mg/kg or 100 mg/kg) were given, the drug (31.6 or 10 mg/ml) was suspended in 1.0% or 0.5% Tween 80.

5-Hydroxytryptamine and 5-hydroxyindoleacetic acid estimation

5-HT and 5-HIAA in the brains of rats were determined fluorimetrically (Ahtee, Sharman & Vogt, 1970) at various intervals after treatment with PCPA. Control values were obtained from untreated animals. All rats were anaesthetized with chloroform and decapitated. The brain was quickly removed from the skull and the pineal body and olfactory lobes were dissected away. 5-HT and 5-HIAA were estimated in the precollicular forebrain.

Alcohol preference test

Rats were tested for alcohol preference using a two-choice, three position method as described by Myers & Holman (1966). The rats were given three graduated drinking tubes, one of which contained water, one alcohol and one was left empty. The position of the tubes was changed at random to counteract any position preference which the rats might develop for the tubes regardless of which fluid they contained. Ethanol solutions were prepared by dilution of 99.9% ethanol with tap water. The ethanol concentrations (v/v) were increased over eight consecutive days from 3 to 25% (3, 5, 7, 9, 11, 15, 20, 25%). Animals were offered only water for three days between each complete eight day testing sequence. No stress was used to induce the rats to drink alcohol, the test was only on voluntary alcohol consumption.

Brain 5-hydroxytryptamine and 5-hydroxyindoleacetic acid when no alcohol was offered

The effects of PCPA given orally or intraperitoneally during eight days were compared in 36 rats divided into three groups. One group ($n = 12$) was given up to eight daily doses of PCPA (316 mg/kg) by stomach tube and four animals were killed for 5-HT assays on days 3, 5 and 9 of the experiment. In the second ($n = 16$) and third ($n = 8$) groups PCPA was administered intraperitoneally; group 2 received the lower dose regimen of 316 mg/kg on the first day and an additional 100 mg/kg four days later; group 3 received the higher dose regimen of 316 mg/kg on the first, fourth and seventh days. 5-HT was estimated for group 2 on days 3, 5, 7 and 9 and for group 3 on days 5 and 10.

Brain 5-hydroxytryptamine and 5-hydroxyindoleacetic acid during alcohol preference tests

Cerebral 5-HT and 5-HIAA were assayed in rats ($n = 12$) tested for alcohol preference before and during the lower dose regimen of intraperitoneal injections of PCPA. All animals in this group were given a baseline eight day preference test at the end of which four animals were killed for 5-HT and 5-HIAA estimations. PCPA was administered to the remaining animals on day 2 of the three 'water-only' days and four days later during the second test sequence. 5-HT and 5-HIAA were determined on the fourth and eighth day after the first injection.

The effect of intraperitoneal injections of p-chlorophenylalanine on voluntary alcohol consumption

This was determined in four groups of eight animals. Following the baseline eight-day preference test two groups were injected with PCPA using the lower dose regimen. The first group of rats were injected with PCPA in 1.0% Tween 80; all following tests were carried out with PCPA

suspended in a 0.5% Tween 80 solution. A third group received PCPA on the higher dose regimen. In all three groups drug treatment began on the second 'water-only' day. The fourth group, a vehicle control group, received 0.5% Tween 80 in the same volume and at the same time intervals as the animals treated on the lower dose regimen. Brain 5-HT and 5-HIAA concentrations were estimated at the end of the three alcohol preference test sequences.

Results

Brain 5-hydroxytryptamine and 5-hydroxyindoleacetic acid concentrations following p-chlorophenylalanine administration

The results of oral and intraperitoneal administration of PCPA on brain 5-HT and 5-HIAA are shown in Table 1. Three days after a single intraperitoneal injection or two successive oral doses of PCPA (316 mg/kg), the cerebral 5-HT content was significantly reduced to 8.4% ($P < 0.01$) and 10.6% ($P < 0.01$) of the control value respectively. This 5-HT depletion was not different in the two modes of administration. Two additional days of oral administration reduced the 5-HT values to 3.6% of control. At this time animals on the lower intraperitoneal PCPA dose regimen had had no further drug treatment and the cerebral 5-HT content was 10.3% of control. However, with the higher dose regimen, the 5-HT concentration was 5.7% of the control, a value not different from that of the orally treated group, but significantly less than that after the lower dose regimen ($P < 0.05$). Twenty-four hours after the eighth and final oral dose of PCPA, the cerebral 5-HT content was found to be 3.0% of the control. This was significantly less than after the lower intraperitoneal dose regimen (17% of control; $P < 0.001$), but not different from the values after the high dose intraperitoneal regimen (4.7% of control). In all cases in which 5-HIAA was measured after PCPA treatment, the acid metabolite was reduced and was often not detectable.

Table 2 shows the results of the lower intraperitoneal dose regimen of PCPA and of Tween 80 control injections on the 5-HT and 5-HIAA concentrations during alcohol preference testing. Immediately following the first alcohol preference test, the 5-HT and 5-HIAA values did not differ from those of controls. PCPA treatment significantly reduced the brain concentrations of 5-HT and 5-HIAA during the second alcohol preference test. At the conclusion of the third preference test the concentration of 5-HT was within the normal range, while that of 5-HIAA was significantly greater than the control value ($P < 0.01$). At the end of the three alcohol preference test sequences animals injected only with 0.5% Tween 80 had cerebral 5-HT and 5-HIAA concentrations similar to those of untreated animals.

Table 1 Effects of intraperitoneal and oral *p*-chlorophenylalanine (PCPA) administration on rat brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) content in rats drinking only water

Days of experiment	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
i.p. PCPA (mg/kg)	316				100					
5-HT			37 ± 6*		45 ± 1*		36 ± 1*		74 ± 6*	
			(8.4)		(10.3)		(8.2)		(17.0)	
i.p. PCPA (mg/kg)	316			316			316			
5-HT					25 ± 8*					20 ± 3*
					(5.7) $n = 8$					(4.7) $n = 8$
5-HIAA					<10*					<10*
oral PCPA (mg/kg)	316	316	316	316	316	316	316	316		
5-HT			45 ± 3*		16 ± 2*				13 ± 4*	
			(10.6)		(3.6)				(3.0)	
5-HIAA			69 ± 2*		<10*				<10*	
			(36.5)							

Each 5-HT and 5-HIAA value is the mean with s.e. mean of four estimations (except where specified) expressed as ng/g brain wet weight and corrected for recovery. Control values: 5-HT, 436 ± 25 ng/g (17 estimations); 5-HIAA, 190 ± 14 ng/g (18 estimations).

* Significantly ($P < 0.01$) different from control value.

Numbers in parentheses are the mean estimates expressed as percentages of the control values.

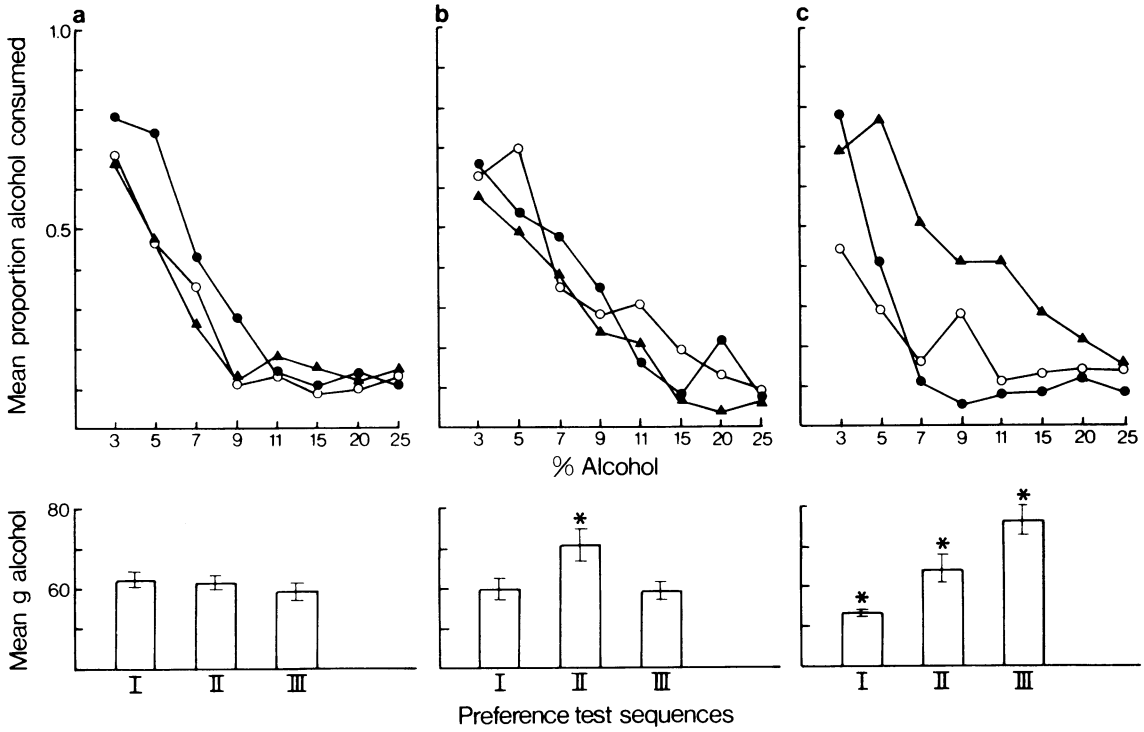

Figure 1 Upper panels: mean proportion of alcohol solution drunk to total daily fluid intake for increasing concentrations of alcohol offered during three preference test sequences: (•) 1st sequence; (○) 2nd sequence; (▲) 3rd sequence. Each point represents the mean for eight animals.
Lower panels: mean amount of alcohol consumed (in g) during each preference test sequence. Each histogram represents a mean of 64 observations. Vertical lines show s.e. mean. (a) 0.5% Tween 80, injected twice and in proportionate volume as in b (vehicle control). (b) *p*-Chlorophenylalanine (PCPA) in 0.5% Tween 80, 316 mg/kg and 100 mg/kg four days later. (c) PCPA in 0.5% Tween 80, 316 mg/kg x3 at three day intervals. In each case the initial injection was given one day before alcohol preference test sequence II began, when only water was offered.
Upper panels: only the 3rd sequence (▲) values in (c) are significantly different ($P < 0.01$) from other test sequences within the series.
Lower panels: * indicates significant difference ($P < 0.01$) from other test sequences within each series of three.

Table 2 Effects of intraperitoneal administration of *p*-chlorophenylalanine (PCPA) and 0.5% Tween 80 on rat brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) during alcohol preference testing

		End of Test I	*Test II Day 3*	*Test II Day 7*	*End of Test III*
PCPA†	5-HT	463 ± 17	29 ± 4	49 ± 6	510 ± 23
	5-HIAA	192 ± 7	<10	16 ± 1	297 ± 22
0.5% Tween 80†	5-HT				393 ± 15
	5-HIAA				164 ± 8

Each 5-HT and 5-HIAA value is the mean with s.e. mean of four estimations, expressed as ng/g brain wet weight and corrected for recovery.
†PCPA, 316 mg/kg, was administered i.p. on the second day of the three day interval between the alcohol preference test sequences I and II. In this interval the rats were given only water to drink. A second dose of PCPA, 100 mg/kg, was administered four days later, i.e. on the third day of the second alcohol preference test sequence.
0.5% Tween 80 (i.p.) was administered on the same time course in a volume of 10 ml/kg per injection.

Alcohol consumption

Figure 1 shows the mean proportion of total daily fluid consumed as an alcohol solution (upper panel), and mean alcohol intake expressed in grams (lower panel) during three test sequences. Control animals treated with 0.5% Tween 80 only (Figure 1a) showed no change in either the proportion of alcohol or grams of alcohol consumed before, during or after the intraperitoneal injections. Figure 1b shows the effect on alcohol consumption of the lower intraperitoneal dose regimen of PCPA in 0.5% Tween 80. In the second test sequence, during the period of drug treatment, the mean proportion of the daily fluid intake taken in the form of alcohol solution was not significantly different from that of the pre- and post-drug sequences. However, during the second test sequence, the average daily fluid intake increased so that the mean total amount of alcohol consumed by the rats did increase to a significant ($P < 0.05$) extent. A rise in the weight of alcohol consumed, but not in the proportion of alcohol solutions consumed was also obtained in a group of eight rats in which 1% (instead of 0.5%) Tween 80 was used to suspend the PCPA.

With the higher dose regimen of PCPA (Figure 1c), the mean proportion of fluid consumed as alcohol was the same in the tests before and during PCPA treatment, but increased significantly ($P < 0.01$) during the post-drug preference test. The mean intake in grams of alcohol increased significantly with each successive sequence ($P < 0.01$).

The results were tested statistically using an analysis of variance.

Discussion

The present results show that two or three intraperitoneal injections of PCPA separated by up to four days can cause as large a depletion of cerebral 5-HT and 5-HIAA as eight successive daily doses given orally. Intraperitoneal administration reduced the number of treatments given to each animal and was not accompanied by any reduction of food and water intake or by weight loss. In fact, the total intake of fluid was increased following the intraperitoneal injection of PCPA. This may reflect an attempt to dilute the PCPA in the peritoneal cavity. However, the volume of alcohol solution consumed expressed as a proportion of the total daily fluid intake remained similar to that observed during the pre-drug testing sequence despite the increased total fluid intake. Only in the period following the high dose regimen of PCPA was there a significant increase in the proportion of alcohol solution consumed.

Although there was a similar depletion of cerebral 5-HT following both oral and intraperitoneal administration of PCPA, no decrease in the selection of alcohol was observed. This suggests that depletion of cerebral 5-HT is not responsible for the changes in alcohol selection reported by Myers & Veale (1968). The difference between the present findings and the results presented by Myers & Veale (1968) appear to be related to the route and time of administration of PCPA and might be explained by the 'conditioned aversion' that occurs when an unpleasant stimulus becomes associated with a rewarding condition, so that the reward is then avoided. In this case, the irritant effects of orally administered PCPA produced avoidance of the alcohol which the rats had chosen to drink before treatment. That repeated oral administration of PCPA has deleterious effects is demonstrated by the fall in body weight reported by Myers & Veale (1968), an observation which was confirmed by our own experiments. Such learned associations have been reported by Nachman (1970) and by Green & Garcia (1971) to occur when milk, grape juice or saccharin was presented to rats prior to treatment with lithium chloride or apomorphine. Nachman *et al.* (1970) found that when rats were injected with PCPA within 5 min of a baseline test for intake of 6% alcohol solution there was a decrease in their subsequent consumption of alcohol.

Similar results were obtained when *n*butyraldoxime, pyrazole or lithium chloride was injected in place of PCPA and when the drugs were tested against the consumption of saccharin solution. These authors suggested that the rats avoided drinking because of an association of the irritant effects of the drugs with drinking. Green & Garcia (1971) have emphasized the importance of the temporal association between the injection of the drug and the presentation of the test solution in the development of such a learned aversion. A solution which was offered before and just after an injection of apomorphine was later rejected by rats, whereas the intake of a fluid which was offered during recuperation from the effects of the injection showed an increase over baseline values. A delay of 30 min between the injection and the presentation of the test fluid could still cause this effect.

In the present experiments there was a 48 h delay between the initial injection of PCPA and the start of the alcohol preference test. This may have prevented the rats from associating the presentation of alcohol with treatment with PCPA.

R.B.H. was supported by a Wellcome Trust Fellowship. We are grateful to Pfizers Limited for generous supplies of parachlorophenylalanine.

References

AHTEE, L. & ERIKSSON, K. (1972). 5-Hydroxytryptamine and 5-hydroxyindoleacetic acid in brain of rat strains selected for their alcohol intake. *Physiol. Behav.*, **8**, 123-126.

AHTEE, L., SHARMAN, D.F. & VOGT, M. (1970). Acid metabolites of monoamines in avian brain; effect of probenecid and reserpine. *Br. J. Pharmac.*, **38**, 72-85.

CICERO, T.J. & HILL, S.Y. (1970). Ethanol self-selection in rats: a distinction between absolute and 95 percent ethanol. *Physiol. Behav.*, **5**, 787-792.

FREY, H.H., MAGNUSSEN, M.P. & NIELSEN, C.H. (1970). The effect of *p*-chloroamphetamine on the consumption of ethanol by rats. *Archs int. Pharmacodyn. Thér.*, **183**, 165-172.

GREEN, K.F. & GARCIA, J. (1971). Recuperation from illness: flavor enhancement for rats. *Science*, **173**, 749-751.

KOE, B.K. & WEISSMAN, A. (1966). *p*-Chlorophenylalanine. A specific depletor of brain serotonin. *J. Pharmac. exp. Ther.*, **154**, 499-516.

MYERS, R.D. (1972). Brain mechanisms involved in volitional intake of ethanol in animals. In *International Symposium, Biological Aspects of Alcohol Consumption*, ed. Forsander, O. & Eriksson, K. *The Finnish Foundation for Alcohol Studies*, **20**, 173-184.

MYERS, R.D., EVANS, J.E. & YAKSH, T.L. (1972). Ethanol preference in the rat: interactions between brain serotonin and ethanol, acetaldehyde, paraldehyde, 5-HTP and 5-HTOL. *Neuropharmacology*, **11**, 539-549.

MYERS, R.D. & HOLMAN, R.B. (1966). A procedure for eliminating position habit in preference-aversion tests for ethanol and other fluids. *Psychon. Sci.*, **6**, 235-236.

MYERS, R.D. & VEALE, W.L. (1968). Alcohol preference in the rat: reduction following depletion of brain serotonin. *Science*, **160**, 1469-1471.

NACHMAN, M. (1970). Learned taste and temperature aversions due to lithium chloride sickness after temporal delays. *J. comp. physiol. Psychol.*, **73**, 22-30.

NACHMAN, M., LESTER, D. & LE MAGNEN, J. (1970). Alcohol aversion in the rat: behavioural assessment of noxious drug effects. *Science*, **168**, 1244-1246.

SHILLITO, E.E. (1970). The effect of parachlorophenylalanine on social interaction of male rats. *Br. J. Pharmac.*, **38**, 305-315.

VEALE, W.L. & MYERS, R.D. (1970). Decrease in ethanol intake in rats following administration of *p*-chlorophenylalanine. *Neuropharmacology*, **9**, 317-326.

(Received August 5, 1974.
Revised September 9, 1974)