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EFFECT OF DESIPRAMINE AND PARGYLINE ON BRAIN GAMMA-AMINOBUTYRIC ACID*

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Abstract—In mice, intraperitoneal administration of desipramine (12·5–100 mg/kg) or pargyline (25–200 mg/kg) reliably elevated the brain concentration of gamma-aminobutyric acid in a dose-dependent manner. In addition, both drugs caused hypothermia and a prolongation of sodium barbital induced narcosis. None of those effects of desipramine, but not of pargyline, were seen when the treated animals were placed in high ambient temperature to prevent drug-induced hypothermia.

There has been ample evidence which implicates gamma-aminobutyric acid (GABA) in the process of neurotransmission in mammalian central nervous system (CNS) [1–6]. However, only limited information is available as to the role of brain GABA in the action of CNS drugs. GABA was proposed to be important in the action of several convulsant drugs [7–14], and in the action of drugs against oxygen toxicity [15–16]. GABA was also related to the withdrawal syndrome following prolonged ethanol inhalation [17].

Recently, it was suggested that a deficiency of brain GABA may be associated with mental illness [18] but its relationship to chemotherapy of mental illness is not known. We, therefore, investigated the effect of the commonly used antidepressant drugs, desipramine and pargyline, on brain GABA. Desipramine is known to inhibit uptake of H^3 -GABA by brain slices [19] and pargyline has been reported to increase brain GABA levels *in vivo* [16].

METHODS

Swiss-Albino, random-bred mice (Charles River Farms, Wilmington, Mass.) weighing 25–30 g were used. They were housed in a room with an alternating 12 hr dark–light cycle. Food (Purina Chow) and water were available *ad lib.* until 1 hr before the experiment. The drug solutions were freshly prepared in double distilled water and were injected intraperitoneally in a volume of 2 ml/kg. Narcosis and body temperature were measured in a constant temperature room maintained at 22° or 30°. For the experiment performed at 30°, the mice were acclimated at that temperature for 24 hr prior to any treatment.

Barbital narcosis. Narcosis induced by sodium barbital (300 mg/kg) was measured 1 hr after pargyline administration. The onset of narcosis was considered to be the time interval between the injection of barbital and loss of the righting reflex, whereas the duration of narcosis was defined as the interval between the loss and regaining of the righting reflex. The endpoint of the righting reflex was determined according to the method of Wenzel and Lal [20] using two reflexes within 30 sec.

Rectal temperature. The rectal temperature was measured with a rectal probe thermister and a Tele-Thermometer (model 425 C, Yellow Spring Instrument Co., Yellow Spring, Ohio). The oil-lubricated probe was inserted to a depth of 1–2 cm. Stable readings were obtained within 10 sec of inserting the probe.

Brain GABA. The mice were sacrificed by immersion in liquid nitrogen. Their brains were excised, GABA was extracted twice by homogenizing in 20 volumes of 75% (v/v) ethanol and centrifugation. The supernatants were evaporated to dryness at 60° in a water bath with constant airflow into each vial containing brain extract. The GABA concentrations were assayed spectrophotometrically after incubation with commercially available enzyme, GABASE (partially purified *Pseudomonas fluorescens* extract essentially free of TPNH activity, obtained from Worthington Biochemical Corporation), by the procedure described by Graham and Aprison. Brain tissues from both the control and the experimental animals were analyzed concurrently in order to take into account day to day variation.

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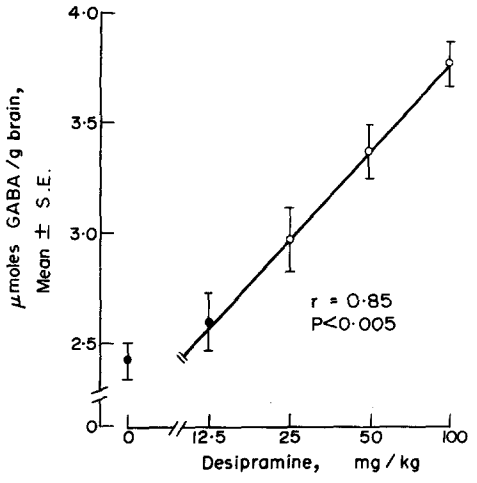

Fig. 1. Effect of various doses of desipramine on mouse brain levels of gamma-aminobutyric acid at 22° ambient temperature. Desipramine was administered 60 min prior to sacrifice. Each point represents the mean ± S. E. from a group of 6 mice for each desipramine dose and seven mice for saline injection (0 desipramine). Open symbols denote significant ($P < 0.05$) difference from controls. P value represents rejection of the hypothesis that the correlation coefficient (r) equals zero.

RESULTS

Effect of desipramine on brain GABA and rectal temperature. Desipramine treatment markedly increased GABA concentration in the whole mouse-brain. Regression analysis of dose–effect relationship showed that the effect of desipramine on brain GABA was reliably dose dependent (Fig. 1), suggesting that the GABA elevation was related to the pharmacological action of this drug. We have previously shown reliable dose-dependent hypothermia in the desipramine treated mice when they are exposed to normal ambient temperature [21]. This hypothermic effect is not seen in mice housed in the environment maintained at 30°. Therefore, we investigated a relationship, if any, between the GABA elevation and the hypothermic effect of desipramine. Data summarized in Table 1 show that the marked elevation of brain GABA seen at room temperature was not present when the mice were housed at 30°. At 22°, desipramine produced hypothermia, but at 30° the hypothermic effect of desipramine was abolished. The analysis of variance on GABA data showed that the brain GABA level depended upon administration of desipramine and ambient temperature. There was also a significant interaction between the desipramine effect and the temperature effect suggesting that the desipramine effect on brain GABA was related to the ambient temperature. The analysis of variance also suggested that changes in ambient temperature themselves were also effective in altering brain GABA levels.

Effect of pargyline on brain GABA and rectal temperature. Similar to the effect of desipramine treatment with pargyline also caused elevation of brain GABA in the mouse. Regression analysis showed that this effect was reliably dose-dependent (Fig. 2). Data given in Fig. 3 show that pargyline caused a dose-dependent decrease in the rectal temperature. Since the effect of desipramine was related to hypothermia, and to the increased sensitivity of brain to barbiturates [21], we investigated in this experiment the effect of pargyline

Table 1. Effect of desipramine on body temperature and brain levels of gamma-aminobutyric acid at two ambient temperatures in mice

	Ambient temp. (°C)	Mean ± S. E. (N) Control*	Treated†
μmoles GABA/g brain	22	2·46 ± 0·08 (6)	3·38 ± 0·14‡ (6)
	30	2·07 ± 0·15§ (7)	2·09 ± 0·13§ (7)
Rectal temp. (°C)	22	38·1 ± 0·2 (7)	34·8 ± 0·3‡ (7)
	30	37·7 ± 0·2 (7)	37·5 ± 0·2 (7)

Source	df	Analysis of variance (GABA data) MS	F	P
Ambient temp. (T)	1	5·394	39·18	< 0·001
Desipramine (D)	1	1·775	12·89	< 0·005
T × D	1	1·666	12·10	< 0·005
Error	24	0·139	—	
	27			

* Distilled water (10 ml/kg) injected intraperitoneally 1 hr prior to sacrifice.

† Desipramine (50 mg/kg) injected intraperitoneally 1 hr prior to sacrifice.

‡ Significantly different ($P < 0.005$) from control, by Student's t-test (two tail). ( ) denotes number of animals in that group.

§ Significantly different ($P < 0.01$) from respective 22° group by Student's t-test.

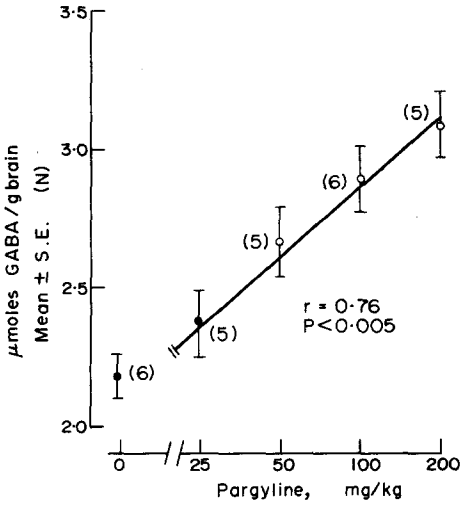

Fig. 2. Effect of various doses of pargyline on mouse brain levels of gamma-aminobutyric acid at 22° ambient temperature. Pargyline was administered 120 min prior to sacrifice. Numbers in parentheses represent number of animals used. Open symbols denote significant ($P < 0.01$) difference from controls. P value represents rejection of the hypothesis that the correlation coefficient (r) equals zero.

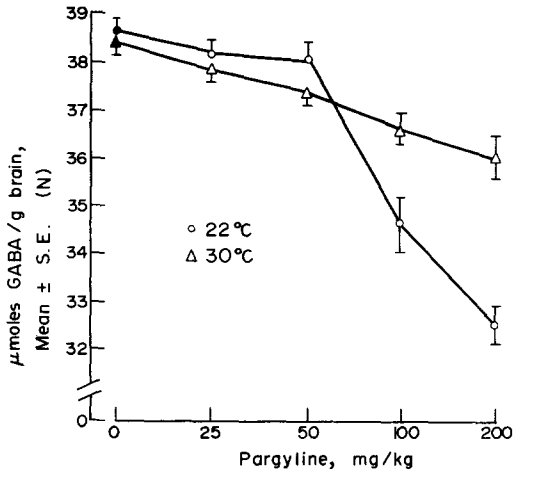

Fig. 3. Effect of pargyline given intraperitoneally on rectal temperature of mice at two ambient temperatures. Pargyline was administered 120 min prior to the measurement of rectal temperature. Each point represents mean ± S. E. of 6 animals. Open symbols denote significant difference ($P < 0.01$) from saline controls.

with respect to those relationships. Barbital narcosis and brain GABA were investigated at both ambient temperatures after pargyline treatment. It is seen from the data summarized in Table 2 that pargyline treatment prolonged barbital narcosis and produced hypothermia at both of the ambient temperatures. However, both of these actions of pargyline were markedly reduced at 30° ambient temperature. Brain GABA was elevated by pargyline at both of the ambient temperatures.

Table 2. Effect of pargyline on barbital narcosis, body temperature and brain levels of gamma-aminobutyric acid at two ambient temperatures

	Ambient temp. (°C)	Mean ± S. E. (N) Control*	Treated†
μmoles GABA/g brain§	22	2·25 ± 0·09 (7)	2·88 ± 0·10‖ (7)
	30	2·29 ± 0·11 (7)	2·79 ± 0·14** (7)
Barbital narcosis (min)‡	22	129 ± 13·1 (7)	261 ± 15·0¶ (8)
	30	102 ± 11·7 (8)	161 ± 8·7¶ (8)
Rectal temp. (°C)	22	38·7 ± 0·1 (6)	34·8 ± 0·5‖ (6)
	30	38·4 ± 0·1 (6)	36·7 ± 0·1‖ (6)

Source	df	Analysis of variance (GABA data) MS	F	P
Ambient temp. (T)	1	0·006	0·068	> 0·05
Pargyline (P)	1	2·228	22·152	< 0·001
T × P	1	0·030	0·300	> 0·05
Error	24	0·100	—	
	27			

* Distilled water (10 ml/kg) injected intraperitoneally 2 hr prior to the measurements.
† Pargyline (100 mg/kg) injected intraperitoneally 2 hr prior to the measurements.
‡ Barbital (300 mg/kg) injected intraperitoneally.
§ Mice were frozen in liquid nitrogen before the determination of brain GABA.
¶ Significantly different controls ($P < 0.001$) by Student's t-test.
‖ Significantly different controls ($P < 0.005$) by Student's t-test.
** Significantly different controls ($P < 0.02$) by Student's t-test.
(N) denotes number of animals in that group.

The analysis of variance showed that the pargyline effect on brain GABA was reliable ($F = 22$, $df = 1, 24$, $P < 0.001$) and, unlike desipramine, it was not dependent upon ambient temperature. Since there was a small degree of hypothermia still present after pargyline treatment at 30°, the role of hypothermia in increasing brain GABA cannot be completely ruled out.

DISCUSSION

Desipramine and pargyline, potent antidepressant drugs, cause marked elevation of brain GABA. Desipramine causes prolongation of narcosis due to sodium barbital and lower brain levels of barbiturates at the time of recovery from narcosis, suggesting that it enhances the neuronal sensitivity to barbiturates [21]. Such effects of desipramine are eliminated if desipramine-induced hypothermia is prevented by elevating ambient temperature. Similar to its effect on neuronal sensitivity, the effect of desipramine on brain GABA is also related to desipramine-induced hypothermia. GABA elevation is seen only in the presence of hypothermia. It may, therefore, be likely that increased neuronal sensitivity to barbiturates caused by desipramine is causally related to the elevation of brain GABA, which may be indirectly due to changes in brain metabolism caused by hypothermia. However, further investigation is necessary to conclusively establish a relationship between neuronal sensitivity (indication of neuronal arousal [21]), brain GABA and body temperature as they are affected by desipramine.

The study with barbital, a barbiturate excreted unmetabolized [22–27], and therefore its duration of action not affected by changes in the hepatic detoxification, suggests that pargyline increases brain sensitivity to barbiturate implying possible reduction in the arousal state. Elevation of brain GABA may be the basis for this change in the arousal state. Unlike desipramine, the pargyline effect on brain GABA is not completely antagonized by high ambient temperature. Therefore, the pargyline effects on neuronal arousal and brain GABA are due to some direct effects of pargyline on brain metabolism and not indirectly caused entirely by hypothermia, although the later mechanism cannot be completely ruled out.

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