The effect of the toxin on seed germination was tested on 4 cultivars of *P. mungo* (L22, H10, B76 and T9) by placing healthy surface-sterilized seeds on filter papers soaked in dilute solution of the toxin in sterile petri dishes. About 90% of the seeds so treated failed to sprout, whereas in the control (without toxin) germination percentage was 90 to 95%. The observation confirms that of Mathur[1].

The toxin caused wilting of cut seedlings of *P. mungo* and tomato. Seedlings (7–10-day-old) were cut under water and transferred immediately to dilute aqueous solution of the toxin in small tubes. Visible wilting started within 1–2 h and cuttings wilted completely within 4–8 h. On placing thin, thoroughly washed beet-root slices in similar toxin solution, the pigment leached out within 15 min indicating that the toxin caused loss of permeability of the cell membrane. On the other hand, intact seedlings did not show any wilting. When surface sterilized seeds of *P. mungo* were grown in dilute sterilized Knop's solution solidified with 1% agar and the toxin was added 3 days after germination, the seedlings showed stunting of growth in comparison to the control, but no wilting. The treated seedlings had also a significant swelling of the base of the hypocotyl.

The treated seedlings showed an increased protein and RNA contents. While the increase in protein content ranged from 5.5 to 10.0% in the different cultivars of *P. mungo*, the total RNA content increased by 9.7–12.2% over the untreated control. Increased protein and RNA contents are known to occur under pathogenic conditions[9,10]. It is of interest to note that similar changes also took place in toxin-treated seedlings, although the changes were of a smaller magnitude.

There were significant increases in the specific activities (enzyme activity/mg protein/min) of the aldolase and the isomerase in the treated seedlings over the control. The aldolase activity increased by 25–55% over control and the isomerase activity, 20–66% in the 4 cultivars studied. The results indicated that the presence of the toxin in the growth medium triggered a higher rate of glucose catabolism by the Embden-Meyerhof pathway. It has been postulated that during actual fungal infection, aldolase activity is stimulated to increase the synthetic rate of phenolic compounds[11]. There was no significant change in the patterns of the isozymes of any of the enzymes studied.

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Pimozide and p-chlorophenylalanine blockade in DL-amphetamine and pargyline-treated rats held at two environmental temperatures

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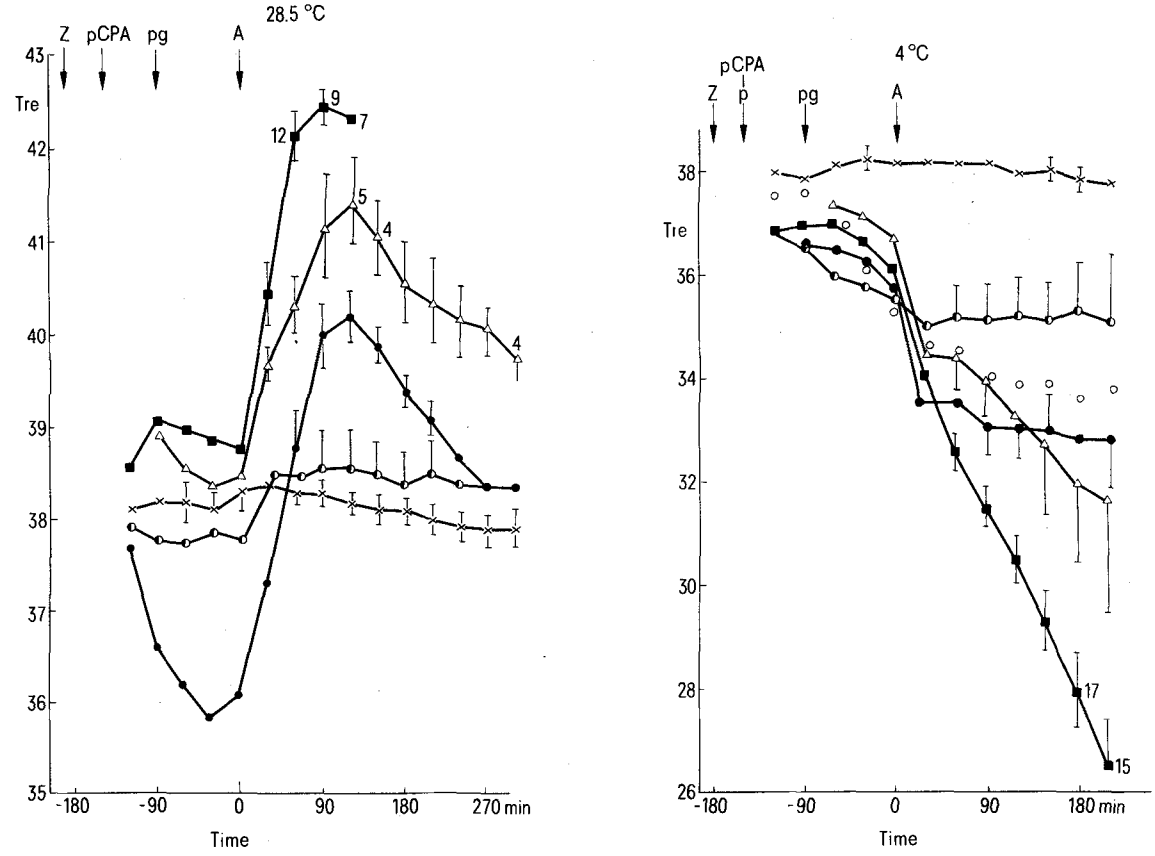
Summary. In rats, treated with DL-amphetamine + monoamine oxidase inhibitor, and held at an ambient temperature of 28.5 °C, hyperthermia was completely eliminated by treatment with pimozide + p-chlorophenyl-alanine. The same drugs markedly reduced the hypothermic effects in rats treated similarly at 4 °C. Results implied that serotoninergic and dopaminergic neurones were involved in the thermoregulatory effects of amphetamine.

Dexamphetamine, a sympathomimetic amine, has effects on body temperature that depend on ambient temperature[1,2]; hyperthermia develops at 20 and 37 °C and hypothermia at 4 °C. These effects are increased by a monoamine oxidase inhibitor. Changes in thermoregulation appear to be related to the release of dopamine in the central nervous system since they are reduced by pre-treatment with pimozide[1–4], a central dopaminergic receptor blocker[5]. However, other neurotransmitters such as 5-hydroxytryptamine (5HT, serotonin) may be involved in the effects of amphetamine[6–8].

The relationship of body temperature and amphetamine treatment in rats pre-medicated with various combinations of a monoamine oxidase inhibitor (pargyline), a depletor of brain 5HT, (DL-p-chlorophenylalanine, pCPA)[9] and pimozide are reported here.

Materials and methods. Male albino Wistar rats (n = 139) weighing 150–200 g were fed and watered ad libitum, and housed in a room at 21 °C. The room was brightly illuminated from 06.00–18.00 h, the rest of the period being dark. Rats of comparable body weights were grouped into 11 groups (n = 4–17) at each experimental temperature, 28.5 or 4 °C. Each experiment lasted 7.5 h and was done using groups of 13 rats comprising 3 controls and 2 treatment groups of 5 rats. Experiments were made on successive days. Pooled data have been used in the illustrations for control rats and in some other cases, e.g. pargyline + amphetamine treatment at 28.5 °C.

Pimozide (Ethnor Pty Ltd, Sydney, Australia) dissolved in 0.05 M tartaric acid in distilled water to a concentration of 1 mg/ml, was given i.p. at a rate of 10 mg/kg b.wt 3 h before amphetamine. DL-p-Chlorophenylalanine hydrochloride (pCPA, Sigma Chemical Co., St Louis, Mo., USA), suspended in 0.05% methyl cellulose solution, was given i.p. at a rate of 125 mg/kg b.wt each day for 3 days, the last dose being given 2 h before amphetamine. Pargyline hydrochloride (Abbott Lab., Sydney, Australia), dissolved in saline was given s.c. at the rate of 25 mg/kg b.wt 90 min before amphetamine. DL-Amphetamine sulphate (Smith, Kline & French, Sydney, Australia), with an optical rotation of zero, was dissolved in 0.09% saline and given i.p. at a rate of 15 mg/kg b.wt immediately after the rats were exposed to the experimental temperatures. Appropriate vehicles were injected into control rats in all experiments when the effects of the various chemicals were tested. Each animal was held in a restraining tube (23 cm long × 7 cm diameter) and rectal temperatures (Tre) were monitored with thermocouples.



Rectal temperature (Tre) of rats held at 28.5 or 4 °C and treated with various drugs. Vertical bars represent SEM. The injections of pimozide(Z), pargyline (pg), the last of 3 doses of p-chlorophenylalanine (pCPA) and amphetamine (A) are indicated by ↓. Times are expressed in relation to injecting amphetamine. Control animals are shown as x—x in all graphs, n = 18 and 19 for 28.5 and 4 °C respectively. Numerals indicate surviving rats. ■ Amphetamine + pargyline, n = 12 (left), 17 (right); △ amphetamine + pargyline + pCPA, n = 6 both graphs; ◐ amphetamine + pargyline + pimozide + pCPA, n = 6 both graphs; ● amphetamine + pargyline + pimozide, n = 6 (left) and 5 (right); ○ pargyline, n = 5.

Results and discussion. 1. 28.5 °C. Treatment with pimozide, pCPA and pargyline separately or together in the 4 possible combinations, either resulted in no change in Tre (pimozide) or caused transitory hypothermia of 0.5–1.5 °C that lasted 3–6 h after treatment. Amphetamine and pargyline, given in combination, caused a rapid and often fatal hyperthermia (figure). However, the magnitude of this hyperthermic effect and the number of mortalities were reduced when in addition either pimozide or pCPA was given; the effects of hyperthermia were completely abolished when these latter drugs were administered together (figure). Although the rats were restricted in their movements, those treated with pimozide, pCPA or both, were markedly quieter than the animals that did not receive these drugs.

2. 4 °C. Tre was reduced by 0.3–1 °C in rats treated with pimozide, pCPA or both and normothermia was restored 3–5 h after treatment. Tre in pargyline-treated rats stabilized at about 34 °C, 2–5 h after treatment (figure). Rats treated with pimozide + pargyline reduced Tre by 2 °C and recovered 5 h after treatment. Tre in pCPA + pargyline-treated rats was reduced to 36 °C after treatment and remained stable for 5.5 h. In rats given pimozide + pCPA + pargyline, Tre was stabilized between 35.5 and 36 °C for 6 h after pargyline injection.

Amphetamine + pargyline lowered Tre by about 10 °C after 210 min exposure, at which time two rats out of 17 collapsed and died (figure). Pre-treatment of similar rats with pimozide (figure) overcame the effects of amphetamine and the residual Tre of about 33 °C was similar to that seen in rats treated with pargyline alone. pCPA was less effective than pimozide in blocking hypothermia in rats treated with amphetamine and pargyline (figure). Pre-treatment of amphetamine + pargyline-treated rats with pimozide + pCPA allowed the animals to stabilize Tre at 35.3 ± 0.27 °C from shortly after amphetamine was given to 200 min cold exposure (figure).

While the results of the present experiments appear to implicate some degree of dopaminergic and serotoninergic neuronal involvement in the thermoregulatory disturbances caused by amphetamine + pargyline, quantitative measurements of the 2 neurotransmitters are necessary to provide conclusive evidence.

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