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The effects of a decarboxylase inhibitor, benserazide, on both thermoregulation and chlorpromazine-induced hypothermia in rats

M. T. LIN*, C. F. CHOW AND Y. F. CHERN

Department of Biophysics and Physiology, National Defense Medical Center, Taipei, Taiwan, Republic of China

Intraperitoneal administration of a peripheral decarboxylase inhibitor benserazide (Ro4-4602) to unanaesthetized rats produced alterations in body temperature which depended on ambient temperature. In the cold, hypothermia was brought about by a decrease in metabolic heat production. At room temperature, a dose-dependent hypothermia was preceded by a slight hyperthermia. The hypothermia was due to an increase in skin temperature (tail) and a decrease in metabolic heat production, while the hyperthermia was due to a decrease in skin temperatures (both tail and footsole) and an increase in metabolic heat production. In the heat, hyperthermia responses to benserazide were associated with decrease in skin temperature (both tail and footsole). Benserazide treatment produced no significant change in brain 5-HT content. Chlorpromazine-induced hypothermia was greatly enhanced after pretreatment of the animals with benserazide at room temperature (22°).

Many reports have shown that systemic administration of peripheral decarboxylase inhibitors, e.g. carbidopa (MK-486) and benserazide hydrochloride (Ro4–4602) which do not enter the brain, decreased the decarboxylation of L-dopa (Chase & Watanable, 1972; Papavasiliou, Cotzias & others, 1972; Rinne, Sonninen & Siirtola, 1972; Mars, 1973) or 5-hydroxytryptophan (Lin, Pang & others, 1978; Gallager & Agahajanian, 1976) in the periphery.

However, to our knowledge, not much information is available on the effects of benserazide on temperature regulation. Also, the possible effect of peripheral decarboxylase inhibition on chlorpromazine-induced hypothermia (Chai, Fann & Lin, 1976; Chai & Lin, 1977) has not been determined. We have investigated these questions with special attention to the dose range and the influence of ambient temperature.

MATERIALS AND METHODS

Eighty-four Spraque-Dawley male rats, 200–250 g, were used. The temperature regulation experiments were on the unanaesthetized animals minimally restrained in special rat stocks. Between experiments the animals were housed individually in wire-mesh cages in a room of 22 ± 1·0° with natural light-dark cycles. The animals were given free access to tap water and granular young chicken feed.

Measurement of thermoregulatory parameters: Rectal (T_{re}) temperature was measured with a copper-constantan thermocouple enclosed in PE 200 tubing, sealed at one end, inserted 6 cm into the rectum. Tail (T_{tail}) and footsole (T_{sole}) skin temperature were also measured using copper-constantan thermocouples. Metabolic rate (M) was calculated from the animals's oxygen consumption and was calculated in watts, assuming an RQ = 0·83 so that one litre of oxygen consumed h^{-1} was equivalent to a heat production of 5·6 W (Stitt, 1973; Lin, 1977; Lin, 1978; Lin & others, 1978). These measurements were made in a small animal partitional calorimeter. All measurements were taken once min^{-1} throughout an experiment, via a Hewlett-Packard digital voltmeter interfaced to an on-line HP 9825 computer. All temperatures and metabolic rate were calculated instantaneously by the computer and displayed by an on-line plotter.

Drug solutions: All glassware was baked at 180° for 5 h before use. All solutions were sterile non-pyrogenic and as an added precaution they were passed through 0·22 μm Swinnex bacterial filters. Drugs administered intraperitoneally included benserazide (N^1-(DL-seryl)-N^2(2,3,4-trihydroxybenzyl) hydrazine, donated by Hoffman-LaRoche Inc., 10–60 mg kg^{-1}); chlorpromazine HCl (cpz, donated by S.K.F. Laboratories, 10 mg kg^{-1}).

Biochemical determination of brain 5-HT (Atack & Lindqvist, 1973), was on the rapidly removed brains of rats treated with benserazide and decapitated 30 min after injection.

RESULTS

Effects of benserazide on temperature regulation

Animals were allowed to acclimatize for at least

* Correspondence.

90 min to the selected ambient temperature (T_a) before drug injections were made. Systematic administration of benserazide produced a two-phase change in body temperature, a slight hyperthermia preceding a more marked hypothermia. The results are summarized in Fig. 1 and described below.

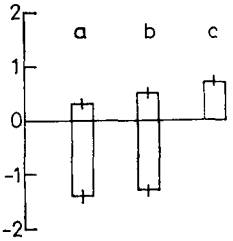

FIG. 1. The maximal changes in rectal temperature (Δ°C) (ordinate) produced by an injection of 40 mg kg^{-1} of benserazide intraperitoneally into 24 unanaesthetized rats at three different ambient temperatures (T_a) of a: 8, b: 22 and c: 31°. n = 8.

In the cold. At a T_a of 8°, the hypothermia developed around 25 min after the injection and T_{re} fell by 1·4 ± 0·14° (Figs 1, 2A). The hypothermia was brought about solely by a decrease in metabolic heat production (M) (Fig. 2A). There were no changes in either T_{tail} or T_{sole}.

At room temperature. A slight hyperthermia developed around 10 min after the injection and T_{re} increased by 0·45 ± 0·13° (Fig. 1). However, the hyperthermia lasted for only 20–30 min and was then followed by a persistent hypothermia (1·3 ± 0·13°) (Fig. 1). The hyperthermia was due to both a decrease in T_{sole} and an increase in M while the hypothermia was due to an increase in T_{sole}, an increase in T_{tail} and a decrease in M (Fig. 2B). Fig. 3 shows the dose-response relation for the hypothermia.

In the heat. At a T_a of 31°, there was an increase in T_{re} (0·7 ± 0·12°) in response to benserazide and both the T_{tail} and the T_{sole} were decreased by benserazide at this T_a (Figs. 1 and 2C). However, metabolic heat production was unaffected.

Effects of benserazide on cpz-induced hypothermia

Administration of cpz (10 mg kg^{-1} i.p.) alone, at room temperature (22°) produced a hypothermia of 2·9 ± 0·31°, while injection of 20 mg kg^{-1} of benserazide alone produced an insignificant change in T_{re}. However, cpz, 10 mg kg^{-1}, i.p., 30 min after prior injection of 20 mg kg^{-1} benserazide produced a greater hypothermia (6·9 ± 0·41°).

Effects of benserazide on 5-HT contents of rat brain

The intraperitoneal administration of doses of benserazide (20–60 mg kg^{-1}) caused no significant change in brain 5-HT contents when compared to control animals during the time at which the thermoregulatory studies were being conducted. Control value 504 ± 36 ng g^{-1} (mean ± s.e. n = 5).

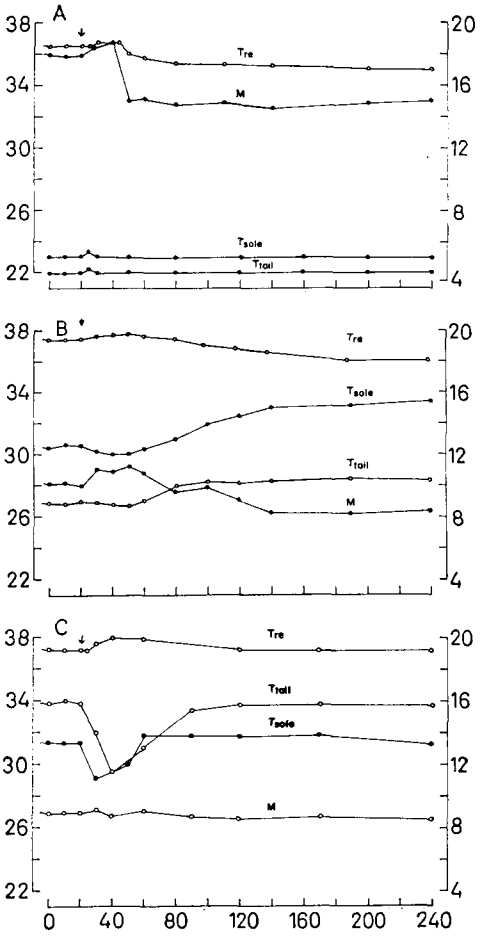

FIG. 2. Thermal responses produced by intraperitoneal injection of 40 mg kg^{-1} of benserazide in an unanaesthetized rat at an ambient temperature (T_a) of A: 8, B: 22 and C: 31°. T_{re} rectal temperature; T_{tail} tail skin temperature; T_{sole} footsole skin temperature and M metabolic heat production. Ordinates left-hand: Rectal and skin temperature (°C); right-hand: Metabolic rate (W kg^{-1}). Abscissa: Time (min).

DISCUSSION

It is now well stated that dihydroxyphenylalanine decarboxylase acts on all naturally occurring aromatic L-amino acids, including dopa, 5-hydroxytryptophan, histidine, tyrosine, tryptophan and phenylalanine. Moreover, potent decarboxylase inhibitors have very little effect on endogenous concentrations of adrenaline and 5-HT in tissue (Cooper, Bloom & Roth, 1974). However, in the present study, the results show that systematic administration of benserazide, a potent peripheral decarboxylase in-

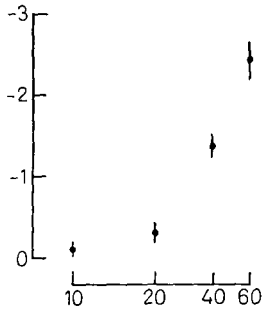

FIG. 3. Dose-response curve for benserazide injected intraperitoneally into 4 groups of 8 animals at an ambient temperature of 22°. The points represent the mean reductions in rectal temperature and the vertical bars denote ± s.e. of the means. Ordinate: Change in rectal temperature (Δ°C). Abscissa: Benserazide (mg kg^{-1}, i.p.).

hibitor, to unanaesthetized rats produced alterations in body temperature which depended on ambient temperature. Therefore, the data suggest that this agent does not act through monoaminergic systems to exert its temperature effect despite the fact that these systems have been repeatedly documented to be involved in temperature regulation (Hellon, 1975).

In addition, the results show that cpz induced hypothermia was greatly enhanced after pretreatment of the animals with benserazide at room temperature (22°). Recently, in this laboratory, the effects of 5-HT changes in brain on hypothermia produced by chlorpromazine have also been studied in unanaesthetized rats at room temperature. For example, specific depletion of 5-HT concentrations both centrally and peripherally with *p*-chlorophenylalanine (PCPA) led to a slight enhancement in cpz hypothermia. Specific depletion of brain 5-HT concentrations with either intracerebroventricular administration of 5,6-dihydroxytryptamine or electrolytic destruction of raphe neurons also resulted in an enhancement in cpz hypothermia. However, replacement of the depleted 5-HT concentrations with 5-hydroxytryptophan administration could not reverse the enhancement of cpz hypothermia produced by PCPA administration. Furthermore, elevating 5-HT concentrations in brain with either 5-hydroxytryptophan alone or 5-hydroxytryptophan in combination with benserazide led to a summation in cpz hypothermia. In addition, elevating 5-HT concentration in 5-HT receptor sites with inhibitors of 5-HT re-uptake also produced synergism in cpz hypothermia. Again, these observations tend to indicate that chlorpromazine does not act through the serotoninergic system to exert its hypothermic effect. However, the data do show that any an inhibition of 5-HT neurons, including electrolytic destruction of 5-HT neurons, inhibition of tryptophan hydroxylase, inhibition of peripheral decarboxylase, inhibition of uptake pump in 5-HT neurons and chemical lesioning of 5-HT neurons, all produced an enhancement in cpz hypothermia.

In summary, the results demonstrate that peripheral decarboxylase inhibition does disrupt the thermal balance and also enhance the hypothermia induced by chlorpromazine in rats.

Acknowledgements

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