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DIFFERENTIAL EFFECT OF BENSERAZIDE ON CATECHOLAMINE CONCENTRATIONS IN THE RAT PINEAL, CEREBRAL CORTEX AND HYPOTHALAMUS

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Abstract—The effect of benserazide, an aromatic L-amino acid decarboxylase inhibitor, has been investigated simultaneously on the noradrenaline content of the pineal, hypothalamus and cerebral cortex of the rat. The dopamine concentration of the hypothalamus was investigated in the same animals. Benserazide had no effect on pineal noradrenaline content throughout the light phase and the early part of the dark phase but caused a drastic reduction in the latter part of the dark period. The drug had no significant effect on noradrenaline or dopamine contents of the hypothalamus. Benserazide treatment caused a large reduction in the noradrenaline content of the cerebral cortex throughout 24 hr. However, this effect is unlikely to be due to a differential penetration of benserazide into the brain areas as a similar degree of decarboxylase inhibition was observed in all three tissues.

In the biosynthesis of melatonin (*N*-acetyl-5-methoxytryptamine: MT), 5-hydroxytryptophan (5-HTP) is converted to 5-hydroxytryptamine under the influence of non-specific aromatic L-amino acid decarboxylase (AAD) E.C. 4.1.1.28. Pineal 5-HT exhibits a diurnal rhythm such that concentrations are high in the day and fall rapidly with the onset of darkness when 5-HT is converted to MT [1]. Thus 5-HT is subsequently *N*-acetylated by *N*-acetyltransferase (NAT) E.C. 2.3.1.5., and then converted to MT by the effect of hydroxyindole-O-methyl transferase (HIOMT) E.C. 2.1.1.4. Both these enzymes exhibit a diurnal rhythm such that dark phase activity is higher than daytime levels [2, 3]. Consequently, serum MT dark phase concentrations are several fold greater than daytime activities. MT synthesis is thought to be induced by darkness which stimulates the release of L-noradrenaline (NA) from postganglionic nerve endings. The neurotransmitter acts on pineal β-adrenergic receptors, thus inducing NAT activity [4] and possibly HIOMT activity [5, 6]. Recently we have shown that benserazide (BZ) (R04-4602), an AAD inhibitor reduces pineal 5-HT by 80% and pineal MT concentrations by 95% whilst leaving hypothalamic 5-HT content unchanged [7]. Surprisingly, we have found that BZ at these doses does not alter NAT activity whilst it seems to abolish the HIOMT rhythm [8]. Since MT after injection elevates hypothalamic 5-HT [9] and concentrates in the rat hypothalamus [10] and since the removal of MT by pinealectomy decreases cerebral cortical NA [11] we report here the simultaneous effect of BZ on the NA content of rat pineal, cerebral cortex and hypothalamus and in the latter case, the effect of the drug on dopamine (DA) content. The AAD activities of these brain regions were also measured.

MATERIALS AND METHODS

Benserazide hydrochloride was donated by Roche Products Ltd (Welwyn Garden City, U.K.). *S*-Adenosyl-L-[methyl-^{3}H]-methionine (specific activity 15 Ci/mmole) and DL-5-hydroxy-[G-^{3}H] tryptophan (specific activity 8.0 Ci/mmole) were purchased from the Radiochemical Centre, Amersham, U.K.

S-Adenosyl-L-methionine, noradrenaline HCl, catechol-O-methyl transferase (E.C. 2.1.1.6), 3-methoxytyramine HCl, normetanephrine HCl, 2-mercaptoethanol, human serum albumin, DL-5-hydroxytryptophan, pyridoxal phosphate and iproniazid were purchased from Sigma Chemical Co., London, U.K. Sodium metaperiodate and glycerol were obtained from the B.D.H. Chemical Co., London, U.K. All other reagents were commercially available and were analytical grade.

The NA was assayed by a modification of the method of Coyle and Henry [12] by the use of commercially available catechol-O-methyltransferase (50 units per assay tube). The assay conditions and the subsequent extraction procedures were identical to the original method. The assay allows the simultaneous measurement of both NA and DA.

Sprague–Dawley rats (180–200 g) were housed in polycarbonate cages and kept in controlled lighting of 14 hr light and 10 hr dark. Water and food were available *ad libitum*. The animals were maintained in this environment for at least one week before experimentation. Twelve groups of rats (N = 5), six experimental and six control, were given i.p. injections of BZ.HCl (80 mg/kg) or the equivalent volume of saline, repeatedly 7, 4 and 1 hr before sacrifice. This dose regimen has been used previously to inhibit pineal and serum MT respectively [7, 8]. Paired groups were sacrificed by decapitation at the following time points 5, 9, 13, 17, 20 and 23 hr after onset of light. The pineal, hypothalamus and the cortex were dissected out and frozen immediately by immersing in liquid nitrogen. The tissues were then stored in dry ice until assay for NA and DA content within two days.

In a separate study, 2 groups of rats housed under

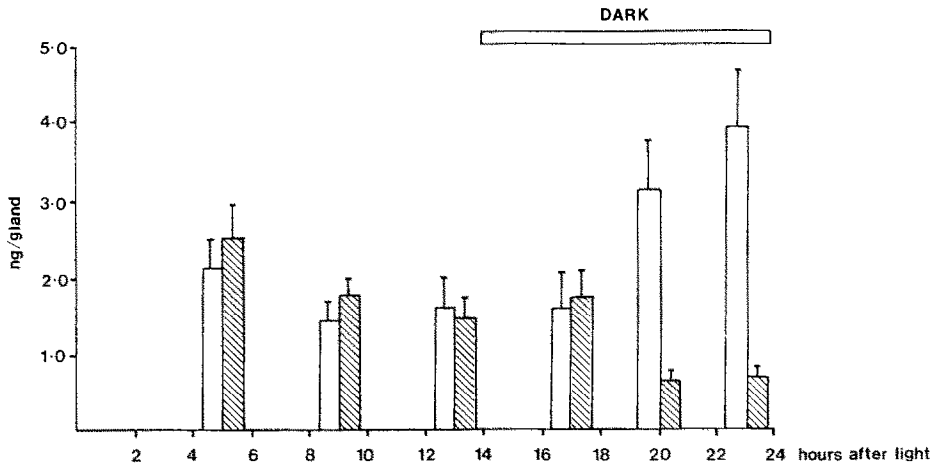

Fig. 1. Effect of benserazide (80 mg/kg) on noradrenaline concentration in rat pineal. Each animal was pre-treated with saline or benserazide HCl (80 mg/kg) 7, 4 and 1 hr before sacrifice at 5, 9, 13, 17, 20 or 23 hr after light (± 10 min). Differences between control (plain columns ± S.E.; N = 5) and drug treated animals (striped columns ± S.E.; N = 5) at 6 and 10 hr dark are significant ($P < 0.002$).

the same condition as before were injected IP with either saline as control or BZ.HCl 80 mg/kg, repeatedly 7, 4 and 1 hr before sacrifice at 7 hr after lights on. The pineal, hypothalamus and cortex were dissected out and frozen as before and later analysed for 5-hydroxytryptophan decarboxylase activity by the method of Snyder and Axelrod [13]. The whole pineal or approximately 10 mg of the hypothalamus and 50 mg of the cortical tissue were used.

RESULTS

Figure 1 shows pineal NA content in male rats throughout 24 hr treated with saline or BZ.HCl (80 mg/kg) repeatedly 7, 4 and 1 hr before sacrifice. The control animals exhibit a diurnal rhythm in their pineal NA content with a maximum 9 hr after onset of darkness and a minimum 9 hr after onset of light. The difference is significant ($P < 0.01$). This rhythm and the levels are similar to those reported by Brownstein and Axelrod [14] using a similar radio-enzymatic assay. However, other workers [15] employing a fluorimetric assay reported higher levels of NA in the pineal. Injection of BZ.HCl (80 mg/kg) repeatedly has no effect on pineal NA content throughout the light phase and the early part of the dark phase. However, during the latter part of the dark phase, injection of the drug causes a significant 75% reduction in pineal NA content at 6 hr after dark ($P < 0.002$).

Figure 2 shows hypothalamic NA concentrations in the same male rats throughout 24 hr treated with saline and BZ.HCl (80 mg/kg) given repeatedly 7, 4 and 1 hr before sacrifice. No statistical difference is seen between the control group and the BZ injected animals at any time point. The concentra-

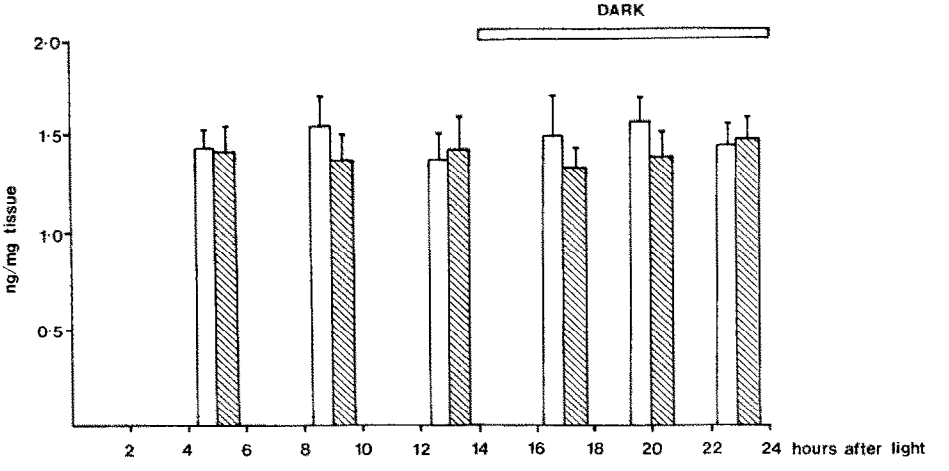

Fig. 2. Effect of benserazide (80 mg/kg) on noradrenaline concentration in rat hypothalamus. Each animal was pre-treated with saline or benserazide HCl (80 mg/kg) 7, 4 and 1 hr before sacrifice at 5, 9, 13, 17, 20 or 23 hr after light (± 10 min). No statistical difference is seen between control (plain columns ± S.E.; N = 5) and drug treated animals (striped columns ± S.E.; N = 5) at any time point.

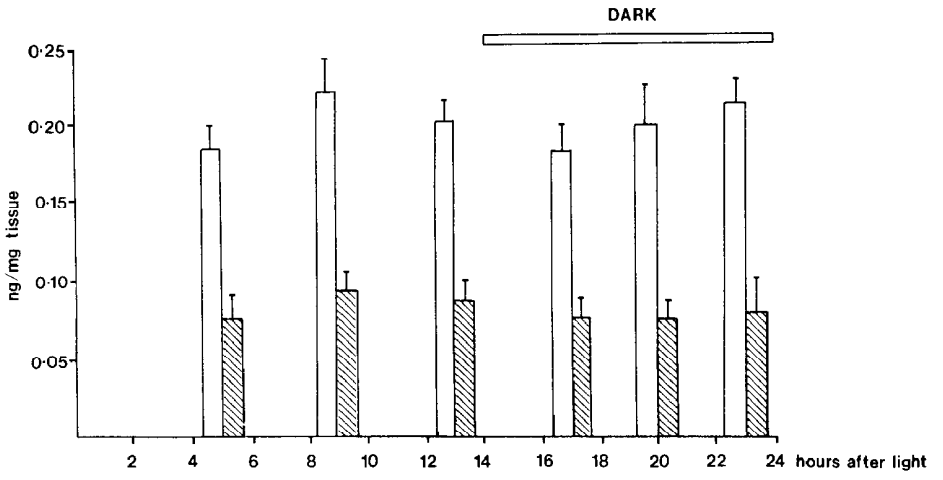

Fig. 3. Effect of benserazide (80 mg/kg) on noradrenaline concentration in rat cortex. Each animal was pre-treated with saline or benserazide HCl (80 mg/kg) 7, 4 and 1 hr before sacrifice at 5, 9, 13, 17, 20 or 23 hr (± 10 min). Differences between control (plain columns ± S.E.; N = 5) and drug treated animals (striped columns ± S.E.; N = 5) are significant at all time points ($P < 0.02$).

tions are similar to those reported [16, 17]. Although some workers report a 24 hr rhythm [16], other workers find no such fluctuation [17]. No statistically significant rhythm is found here.

Figure 3 illustrates the cortical NA in these male rats treated with saline or BZ.HCl (80 mg/kg) repeatedly 7, 4 and 1 hr before sacrifice. The control group showed no statistically significant 24 hr rhythm. The values agree well with those reported [18, 19]. However, injection of BZ causes a significant reduction ($P < 0.02$) at all the time points tested both in the day and night.

Figure 4 show hypothalamic DA content in these male rats treated with saline or BZ.HCl (80 mg/kg) repeatedly 7, 4 and 1 hr before sacrifice. No statistical difference is seen between the control group and the BZ injected animals at any time point. The concentrations are similar to those reported [20].

The DA levels in both the pineal and the cortex were on and below the detection limit of the assay procedure used respectively.

Table 1 summarises the 5-hydroxytryptophan decarboxylase (5-HTPase) activities in the pineal, hypothalamus and the cortex of the control and injected rats. The enzyme activity is expressed as the radioactivity of the product formed by the enzyme. Under the dosage regimen of BZ used here, a significant reduction of 5-HTPase activities was observed in all the tissue tested. The percentage reductions, although appearing to be highest in the cortex and lowest in the pineal, are of similar magnitude.

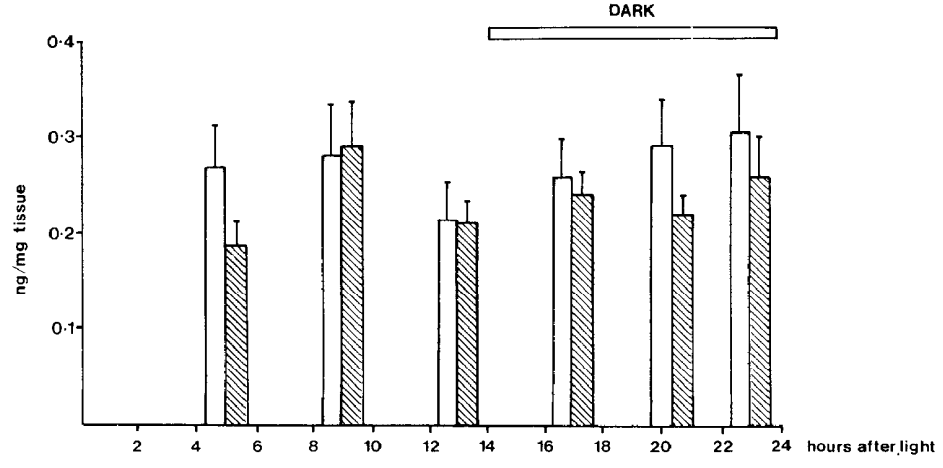

Fig. 4. Effect of benserazide (80 mg/kg) on dopamine concentrations in the rat hypothalamus. Each animal was pre-treated with saline or benserazide HCl (80 mg/kg) 7, 4 and 1 hr before sacrifice at 5, 9, 13, 17, 20 or 23 hr after light (± 10 min). No statistical difference is seen between control (plain columns ± S.E.; N = 5) and drug treated animals (striped columns ± S.E.; N = 5) at any time point.

Table 1. 5-Hydroxytryptophan decarboxylase activities in the pineal, hypothalamus and cortex after repeated injections of BZ.HCl (80 mg/Kg) at 7, 4 and 1 hr before sacrifice at 7 hr after lights on

Tissue	Control animals Mean ± S.E. (N = 5)	BZ injected animals mean ± S.E. (N = 5)	Percentage reduction
Pineal	4139 ± 329	1458 ± 103	(65%)
Hypothalamus	268 ± 13	111 ± 5	(59%)
Cortex	31 ± 3	8 ± 1	(73%)

Values are CPM/pineal gland or CPM/mg of wet tissue of hypothalamus or cortex. All reductions are statistically significant ($P < 0.001$).

DISCUSSION

In the synthesis of monoamines from their corresponding amino acids, the hydroxylation step (by tyrosine hydroxylase E.C. 1.14.16.2. [21, 22] or tryptophan hydroxylase E.C. 1.14.16.4 [23]) is generally accepted as the rate limiting step. Inhibition of the aromatic amino acid decarboxylase (AAD) would not normally reduce the monoamines synthesis. In the case of the pineal, however, the rate-limiting step is more difficult to define, as it has been demonstrated that AAD inhibition can lead to 80% reduction in its 5-HT content throughout the 24 hr day/night cycle [7]. The situation is further complicated by the present study which shows that AAD inhibition has little effect on NA concentrations during the day and earlier part of the night, but significantly reduces the pineal NA content during the latter part of the night.

It is possible that BZ can only influence the synthesis of monoamines in the pineal when their turnover rate is high. It has been demonstrated that pineal 5-HT has a very high turnover rate even during the daytime when it is supposed to accumulate in the pineal [24]. The turnover rate of NA has also been shown to double during the latter part of the night [25]. The lack of effect of BZ on the daytime pineal NA level may reflect the fact that the slower hydroxylation step is still rate limiting. However, with the tyrosine hydroxylase activity increasing during the dark phase [26], a reduced decarboxylase activity (by BZ) may in turn become rate limiting. This, coupled with the higher turnover rate of NA, may result in lowering the pineal NA content at night as observed here. A similar differential effect has recently been suggested in pineal melatonin regulation [27]. During the daytime and early part of the night, NAT is rate limiting. As the NAT activity rises in the later part of the night, HIOMT becomes rate limiting. The present result demonstrates that the responsiveness of the pineal to pharmacological manipulation may depend on the time of the light/dark cycle when the agent is administered. Nevertheless, the reduction of NA content in the pineal in the later part of the dark phase does not correlate with the drug's lack of effect on the β-adrenergically controlled NAT at night [8]. Even though BZ reduces the NA content in the later stages of the dark phase, there may be sufficient NA present to act at the pinealocyte postsynaptic receptor to activate the nocturnal surge in NAT activity.

BZ at 50 mg/kg is commonly adopted as a peripherally acting agent, with minimal effect on cerebral decarboxylase activity [28, 29]. Even at 500 mg/kg, whilst substantial inhibition of decarboxylase activity occurred, when total brain catecholamine content was measured, no significant reduction was found [30]. However, in the present study, although there was no effect by BZ on the hypothalamic catecholamine content, a significant reduction in cortical NA was observed. Relative to the pineal, both the hypothalamus and the cerebral cortex are within the blood brain barrier (BBB) though the hypothalamus is considered to have an incomplete barrier [31]. However, this relative position to the BBB cannot explain the differential effect of BZ in the two central brain areas. Indeed when the 5-HTPase activity was measured after the drug treatment, a similar degree of decarboxylase inhibition was observed. This result suggested that BZ penetrates into the hypothalamus and cortex to the same extent.

Although there is still an existing doubt whether dopa-decarboxylase and 5-hydroxytryptophan decarboxylase are the same enzyme [32], BZ being non-specific in this respect is able to inhibit both [33]. Therefore, the measurement of 5-HTPase should give some indication of the total aromatic acid decarboxylase activity.

One possible explanation for the differential effect of BZ on the hypothalamic and cortical NA, could be the difference in their relative content of the AAD. The hypothalamus contains a much higher concentration of the enzyme than that in the cerebral cortical area [34]. This relative enzyme concentration is also in accordance with the relative tissue content of NA, in these two brain areas as found here (approx. 2 ng/mg in hypothalamus and 0.2 ng/mg in the cortex). It is demonstrated that under the repeated injection regimen adopted here, BZ has penetrated through the BBB and has caused a similar degree of decarboxylase inhibition in all three tissues. However, because of the relatively high decarboxylase content in the hypothalamus, such a level of inhibition may not be significant enough to affect the overall production of NA in this brain area, bearing in mind that the hydroxylation step is normally the rate limiting step in catecholamine synthesis. In the cerebral cortex, a similar degree of

decarboxylase inhibition may have reduced the already low enzyme activity to the extent that it became rate limiting thus reducing the production of NA and producing a fall in NA content.

Thus, it is demonstrated that, although BZ is believed to be peripherally acting when administered under the present dosage regimen, it can evoke in the same animals different responses from the three brain regions tested.

The dopamine concentrations in the pineal and cerebral cortex were below the sensitivity of the assay used here. The lack of effect of BZ on the hypothalamic DA is perhaps in accordance with the drug's inability to reduce the hypothalamic NA.

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