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Pineal Gland as a Model to Elucidate the Primary Mode of Action of Alpha-Methyldopa: Alpha-Methyldopa Induces an Increase in the Synthesis of N-Acetylserotonin and Melatonin Levels by the Rat Pineal Gland

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An attempt was made to use the pineal gland as a model for the study of the primary mode of action of alpha-methyldopa, which is still unclear. Organ cultures of pineal glands from rats treated chronically with alpha-methyldopa showed enhanced conversion of radio-active serotonin to melatonin (aMT) as well as its precursor, N-acetyl-serotonin (aHT). This treatment was also found to raise serotonin-N-acetyl-transferase (NAT) activity. These increases associated with alpha-methyldopa treatment were further enhanced by the beta-adrenergic agonist, isoproterenol, suggesting a supersensitivity-type effect occurring at the level of the beta-receptor. A subsequent binding study, however, showed a decrease in beta-receptor binding with exposure to alpha-methyldopa, providing mitigating evidence against the occurrence of a supersensitivity phenomenon. It is possible that a metabolite of alpha-methyldopa acts as an alpha 1 and beta-agonist, resulting in greater melatonin (aMT) and N-acetylserotonin (aHT) synthesis than by a beta-agonist, isoproterenol.

Key words: serotonin, adrenoreceptors, N-acetyl-transferase, alphamethylnorepinephrine

INTRODUCTION

Melatonin is synthesized in the pineal gland by acetylation of serotonin (5HT) to form N-acetylserotonin (aHT) by N-acetyltransferase (NAT). The enzyme hydroxyindole-O-methyltransferase (HIOMT) subsequently converts N-acetyl-serotonin (aHT) to melatonin (aMT) (Ebadi et al., 1986).

In the rat, the synthesis of melatonin may be regulated transsynaptically by the release of norepinephrine, which interacts with beta-adrenergic receptors on pinealocytes. This interaction results in the activation of membrane-bound adenylate cyclase, the increased formation of 3′,5′-cyclic adenylic acid (cAMP), and the increased synthesis of a specific protein involved in the induction of N-acetyltransferase (NAT) (Ebadi et al., 1986). Studies by Alphs et al. (1980) and Klein et al. (1983) provide evidence that alpha-receptors are also involved in the stimulation of melatonin formation.

The responsiveness of a pinealocyte depends upon prior exposure of the beta-adrenergic receptors of the cell to norepinephrine. Abolition of the noradrenergic activity by denervation, or reduction by reserpine, results in a great enhancement in the sensitivity of the beta-adrenergic receptors. If the number of catecholamine molecules reacting with beta-adrenergic receptors is increased for a period of time, the pineal beta-receptors become less responsive to the agonist (Deguchi and Axelrod, 1972). These phenomena are referred to respectively as "super- and subsensitivity."

Alpha-methyldopa (levo-3-(3,4-dihydroxyphenyl)-2-methylalanine) is an antihypertensive agent currently in clinical use, which is thought to exert its effect within the central nervous system. This drug is converted in vivo to alpha-methylnorepinephrine, which has been speculated to act as a false transmitter in place of norepinephrine (Rudd and Blaschke, 1985). The primary mode of action of alpha-methyldopa needs further elucidation. Since the pineal gland has a well-characterized metabolic pathway which is linked to both alpha- and beta-receptors, we decided to investigate the primary mode of action of alpha-methyldopa by using the gland as a model.

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MATERIALS AND METHODS

Chemicals

Alpha-methyldopa, ±isoproterenol, and propanalol were obtained from Sigma Chemical Co. (St. Louis, MO). BGJb culture medium was obtained from Gibco (Europe). Chromatography plates (Kieselgel $60F_{254}$) were obtained from Merck (West Germany). The 5-hydroxy-[side chain-2-^{14}C] tryptamine creatinine sulfate (specific activity : 57 mCi/mmol),[^{3}H]-acetyl-coenzyme A (specific activity: 4.0 Ci/mmol), and [^{3}H]- CGP-12177 (4-(3-t-butylamino-2-hydroxy-propoxy)-[5,7-^{3}H] benzimidazol-2-one) (specific activity 30 Ci/mmol) were obtained from Amersham (England).

Animals

Albino male rats of the Wistar strain were used and maintained under an automatically regulated lighting cycle of LD 12:12 in well-ventilated housing at a constant temperature of 20°C.

Methods

For all the experiments undertaken, the animals were divided into groups of five animals each, with one group serving as the control. In the instances where alpha-methyldopa was injected, it was given i.p. at a concentration of 50 mg/kg over a 1½ day period (1000 hr, 1700 hr, and 1000 hr). Alpha-methyldopa was dissolved in deionized, distilled water, and fully solubilized with 0.05% HCl. Animals in the control group received the vehicle for alpha-methyldopa. Organ culture experiments and separation of [^{14}C]-serotonin metabolites (Klein and Notides, 1969) were performed in order to determine the effect of alpha-methyldopa on the pineal metabolites aHT and aMT. Individual pineal glands were collected and incubated in BGJb culture medium (60 μl) containing 2 μCi 5-hydroxy-[side chain-2-^{14}C]tryptamine creatinine sulfate (specific activity: 57 mCi/mmol) and incubated for 24 hr in a 5% CO_2:95% O_2 environment with a 95% humidity. After 24 hr, the incubation was terminated by removal of the pineal glands from the culture medium. A 10 μl aliquot of the culture medium was removed for isolation of the radioactive pineal metabolites by two-dimensional thin-layer chromatography. The [^{14}C]-aHT and [^{14}C]-aMT in the 10 μl samples were measured by liquid scintillometry. Pineal [^{14}C]-aHT and [^{14}C]-aMT levels were determined after administration of alpha-methyldopa to rats. The two metabolite levels were then determined after in vitro exposure of pineal glands to 10 μM alpha-methyldopa. Levels were also determined after in vitro stimulation with isoproterenol. In another experiment, the metabolite levels were also determined after isoproterenol stimulation of pineal glands from alpha-methyldopa-treated rats.

The assay used for the determination of NAT was a modification of the method described by Deguchi and Axelrod (1972). Tryptamine (0.1 mmol) and [^{3}H]-acetyl-coenzyme A (0.125 μCi) were added to 50 μl pineal homogenate and incubated at 37°C for 10 min. The reaction was terminated with 0.5 ml borate buffer (pH 10). The [^{3}H]-N-acetyltryptamine was extracted into an organic phase (toluene:isoamyl alcohol 97:3) and the radioactive N-acetyltryptamine formed was measured by liquid scintillometry. NAT activity was determined after administration of alpha-methyldopa, stimulation with isoproterenol, and after isoproterenol stimulation of pineal glands from alpha-methyldopa-treated rats.

Binding study

The effect of alpha-methyldopa on beta-adrenergic receptor binding in rat pineals was investigated by using a modification of a technique by Wilkinson and Wilkinson (1985). Pineal glands from an alpha-methyldopa-treated group and from a control group were bisected and placed each in 225 μl phosphate-buffered saline (pH 7.4). Propanalol (10 μM) was added to one half of each bisected pineal from each group for determination of nonspecific binding. The ligand [^{3}H]-CGP-12177 (4-(3-t-butylamino-2-hydroxypropoxy)-[5,7-^{3}H]-benz-imidazol-2-one)(0.0075 μCi) was added to each tube, and the samples were incubated at 37°C for 120 min. The half-pineal glands were then removed and placed in 2 ml emulsifier and scintillation cocktail and [^{3}H]-CGP levels were measured by liquid scintillometry. Specific binding was defined as total binding minus nonspecific binding. Nonspecific binding was not greater than 25% of total binding.

STATISTICS

Statistical comparisons were made by using Student's t test. Data derived from the organ culture experiments are expressed as dpm (×10) [^{14}C]-serotonin metabolite/10 μl medium/pineal gland. NAT activity is expressed as fmoles product/gland/hr and specific binding as cpm [^{3}H]-CGP/half-pineal gland. All data referred to were calculated as the mean ± S.E.M. (n=5).

RESULTS

Pineal glands from rats treated with alpha-methyldopa produced significantly elevated levels of aHT ($P<0.001$) as well as aMT ($P<0.025$) (Figs 1b, 2b) in comparison to basal values. However, in vitro exposure of pineal glands to alpha-methyldopa did not alter the levels of these metabolites. Exposure of pineal glands to

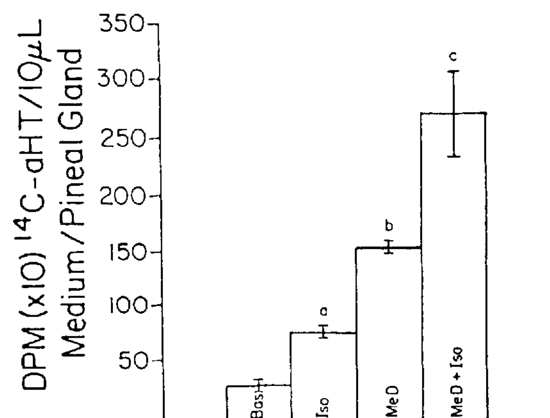

Fig. 1. Effect of isoproterenol, alpha-methyldopa, and isoproterenol and alpha-methyldopa on rat pineal gland production of N-acetylserotonin (aHT) ± S.E.M. **a:** $P<0.001$ vs. basal levels. **b:** $P < 0.001$ vs. basal levels. **c:** $P<0.025$ vs. alpha-methyldopa treatment. Bas = basal, Iso = isoproterenol, MeD = alpha-methyldopa.

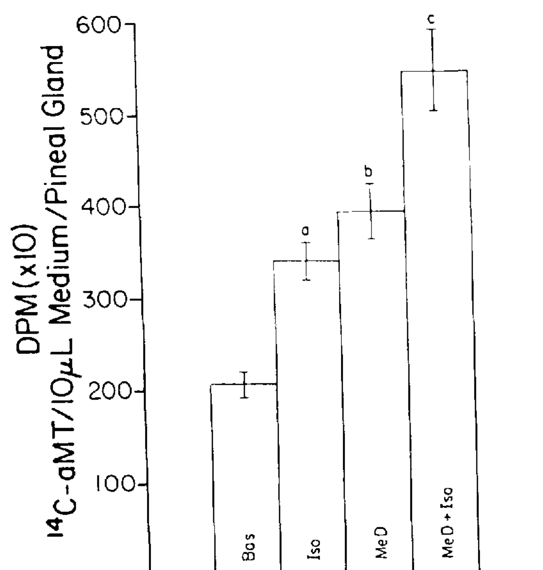

Fig. 2. Effect of isoproterenol, alpha-methyldopa, and isoproterenol and alpha-methyldopa on rat pineal gland production of melatonin (aMT) ± S.E.M. **a:** $P < 0.001$ vs. basal levels. **b:** $P < 0.025$ vs. basal levels. **c:** $P < 0.025$ vs. alpha-methyldopa treatment. Bas = basal, Iso = isoproterenol, MeD = alpha-methyldopa.

isoproterenol (10 μM) led to a significant increase in pineal production of aHT ($P < 0.001$) and aMT ($P < 0.001$) (Figs 1a, 2a) in comparison to basal levels. Pineal gland production of aHT and aMT after exposure of pineals from alpha-methyldopa-treated rats to isoproterenol were significantly raised in comparison to the raised levels obtained after alpha-methyldopa treatment alone ($P < 0.025$).

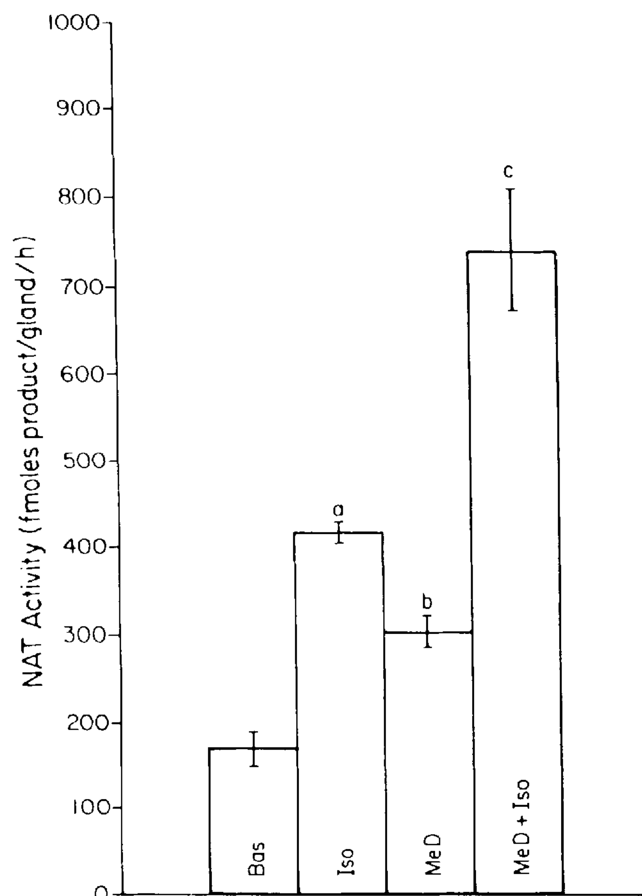

Fig. 3. Effect of isoproterenol, alpha-methyldopa, and isoproterenol and alpha-methyldopa on rat pineal N-acetyltransferase (NAT) activity ± S.E.M. **a:** $P < 0.025$ vs. basal levels. **b:** $P<0.025$ vs. basal levels. **c:** $P<0.025$ vs. alpha-methyldopa treatment. Bas = basal, Iso = isoproterenol, MeD = alpha-methyldopa.

Accordingly, a commensurate rise in pineal NAT activity ($P < 0.025$) was observed when rats were treated with isoproterenol i.p. (Fig. 3a). A significant increase in NAT activity ($P < 0.025$) was also observed with alpha-methyldopa treatment (Fig. 3b). The increase in NAT activity in pineals exposed to a combination of alpha-methyldopa (in vivo) and isoproterenol (in vivo) was significantly greater than that observed in pineals exposed to alpha-methyldopa (in vivo) alone ($P < 0.025$) (Fig. 3c).

The stimulation of the pineal gland with in vivo alpha-methyldopa treatment was accompanied by a significant decrease in pineal beta-receptor binding ($P <0.025$) (Fig. 4).

DISCUSSION

Treatment of rats with alpha-methyldopa resulted in a significant increase in pineal production of aHT and

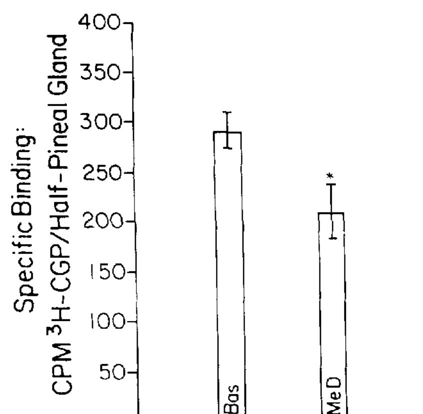

Fig. 4. Effect of alpha-methyldopa on specific binding of rat pineal gland beta-receptors ± S.E.M. $*P<0.025$ vs. basal specific binding. Bas = basal, MeD = alpha-methyldopa.

aMT. In vitro exposure of pineal glands to alpha-methyldopa, however, did not increase the level of these two metabolites, suggesting that either alpha-methyldopa requires to be metabolized to an active metabolite or that it is unable to reach pinealocytes in vitro. The former supports the current theory regarding the fate of alpha-methyldopa in the body that alpha-methyldopa is converted to alpha-methyl-norepinephrine by the action of dopa-decarboxylase. The alpha-methyl-norepinephrine is hypothesized to act as a false transmitter (Rudd and Blaschke, 1985). The postganglionic neurons which terminate in the pineal may be the site of metabolism of alpha-methyldopa. Degeneration of these endings subsequent to removal of the pineal may thus account for the failure of alpha-methyldopa to be converted in vitro to an active metabolite. An alternative consideration is that the in vivo promotion of aHT and aMT production is induced via the sympathetic innervation of the gland following a primary action of the drug in the brain.

The stimulation of pineal glands with isoproterenol resulted in significantly raised levels of aHT and aMT as expected. However, when isoproterenol was used to stimulate pineal glands from rats treated with alpha-methyldopa, levels of aHT and aMT were found to be raised to a significantly greater extent than with alpha-methyldopa treatment alone. This is suggestive of the occurrence of a supersensitivity-type phenomenon at the level of the beta-receptor, as has been shown to occur with reserpine (Cantor et al., 1981). In accordance, we observed raised NAT levels with alpha-methyldopa treatment and the further enhanced levels of the enzyme associated with isoproterenol stimulation of pineal glands from alpha-methyldopa-treated rats. The possibility of alpha-methyldopa inducing supersensitivity of the pineal beta-receptor, making the alpha-methyldopa treated rats more responsive to the beta-agonist isoproterenol, can be ruled out since the binding study showed that alpha-methyldopa treatment reduces ligand binding to the beta-receptor.

A possible explanation of the alpha-methyldopa potentiation of the beta-agonist isoproterenol is that the metabolite of alpha-methyldopa, alpha-methylnorepinephrine, interacts with the alpha-receptor. Melatonin production in the rat pineal is controlled by norepinephrine acting via alpha 1– and beta-adrenoreceptors on pinealocytes (Sugden et al., 1984); activation of alpha-adrenoreceptors appears to potentiate beta-adrenergic stimulation of rat pineal NAT by isoproterenol. Alpha-methylnorepinephrine might possibly also potentiate the beta-adrenergic stimulation of N-acetyltransferase by acting on the alpha-receptor. This might serve to explain the significant increases in aHT, aMT, and NAT activity associated with alpha-methyldopa treatment, as well as the potentiation of the isoproterenol response by alpha-methyldopa.

In conclusion, alpha-methylnorepinephrine is a weak beta-agonist and possibly an alpha-receptor agonist as well. The alpha-agonistic activity of the metabolite of alpha-methyldopa remains to be confirmed by using an alpha-receptor blocking agent. In a broader context, this study also serves to demonstrate that in the pineal gland, the available adrenergic receptors which are coupled to a well-characterized metabolic pathway represent an excellent compartmentalized biochemical system for use in investigations into the primary modes of action of sympathoactive drugs.

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