Increased adrenaline, β-adrenoreceptor stimulation and phospholipid methylation in pineal gland of spontaneously hypertensive rats

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Summary

1. In pineal glands of adult spontaneously hypertensive rats (Okamoto strain), dopamine levels increased 34%, adrenaline concentrations increased 152% and noradrenaline levels decreased by 26%, when compared with Wistar-Kyoto normotensive controls.

2. These results are consistent with the hypothesis of an increased uptake of peripheral adrenaline resulting in increased release of noradrenaline from pineal sympathetic nerves in hypertensive rats.

3. The activity of pineal N-acetyltransferase was increased fourfold in hypertensive rats, indicating increased stimulation of pineal β-adrenoreceptors.

4. Methylation of pineal membrane phospholipids was also increased (100–320%) in hypertensive rats.

5. These results indicate a correlation in vivo between increased β-adrenoreceptor stimulation and increased methylation of membrane phospholipids in the rat pineal gland, which could result in changes in membrane fluidity and affect the coupling of the β-adrenoreceptors to the adenylate cyclase.

Key words: adrenaline, catecholamines, genetic hypertension, β-adrenoreceptors, phospholipid methylation, pineal gland.

Introduction

Peripheral catecholamines play a role in the regulation of blood pressure and in some forms of hypertension (de Champlain, Krakoff & Axelrod, 1966; Nakamura, Gerold & Thoenen, 1971; De Quattro & Alexander, 1974). Spontaneously hypertensive rats (Okamoto, 1972) exhibited increased synthesis of adrenal catecholamines (Grobecker, Saavedra, Roizen, Weise, Kopin & Axelrod, 1976) and increased release of peripheral catecholamines to the circulation when submitted to mild stress (McCarty & Kopin, 1978). This increased release of peripheral catecholamines could result in changes in the stimulation of adrenoreceptors and play a role in the development or maintenance of hypertension.

The pineal gland is a useful organ for studying the relationship between neurotransmitters and responsive cells, and especially the regulation of adrenergic function. This gland is innervated by sympathetic nerves, which in turn stimulate pineal β-adrenoreceptors (β-receptors). The pineal gland responds to β-adrenergic stimulation with a cyclic AMP-mediated increase in the activity of N-acetyltransferase, resulting in increased synthesis of pineal melatonin. Measurement of pineal N-acetyltransferase activity provides an index of the degree of β-receptor stimulation in this organ (Axelrod & Zatz, 1977). In addition to noradrenaline liberated by pineal sympathetic nerves, the pineal β-receptors could be activated by circulating catecholamines, including adrenaline originating in the adrenal medulla (Lynch & Wurtman, 1977).

Stimulation of β-receptors in rat reticulocytes, HeLa cells and astrocytoma cells resulted in increased methylation of membrane phospholipids. Synthesis of phosphatidylcholine occurs in membranes by a stepwise methylation of phosphatidylethanolamine by two methyltransferases. The phospholipid methyltransferases,
their substrates and their products are asymmetrically distributed in the cell membranes. Methyltransferase I, located on the cytoplasmic side of the cell membrane, transfers a methyl group from S-adenosylmethionine to phosphatidylethanolamine to form phosphatidyl-N-monomethyllethanolamine. Methyltransferase II, on the exterior surface of the membrane, catalyses two additional N-methylations to form phosphatidylcholine (Hirata, Viveros, Diliberto & Axelrod, 1978). This asymmetric distribution of methyltransferases results in the synthesis of phosphatidylcholine and in the translocation of the phospholipid from the inside to the exterior surface of the membrane. Phospholipid methylation and translocation increases membrane fluidity, which, by influencing the lateral movement of proteins, could affect the coupling of the β-receptors to the adenylyl cyclase (Hirata, Strittmatter & Axelrod, 1979).

The present study reveals that the pineal gland of spontaneously hypertensive rats (SH rats) contains more dopamine and adrenaline, and less noradrenaline, than the pineal of control rats. In addition, the activity of pineal N-acetyltransferase and the methylation of pineal membrane phospholipids are increased in SH rats.

Methods

Animals

Male 14-week-old SH rats and normotensive Wistar-Kyoto controls (WKY rats) were obtained from Taconic Farms (Germantown, New York, U.S.A.). The animals were housed six per cage for 2 weeks with unrestricted food and water under a 12 h light schedule, with lights on from 06.00 to 18.00 hours.

The systolic blood pressure was measured 2 days before the rats were killed, by a tail cuff plethysmograph pulse transducer and a programmed electrophygmomanometer (Narco Biosystems, Houston, Texas, U.S.A.; model PE-500).

The animals were killed at 10.00 hours and the pineal glands immediately removed, frozen on solid CO₂ and kept frozen at −20°C until assayed.

Biochemical assays

The catecholamine content of pineal glands was measured by the method of Da Prada & Zurcher (1976). Pineal N-acetyltransferase activity was determined by the method of Deguchi & Axelrod (1972).

The production of methylated phospholipids by incubation of pineal membranes in the presence of [³H]methyl-S-adenosylmethionine (New England Nuclear, Boston, Mass., U.S.A.; specific radioactivity 12 Ci/mmol) was measured as reported by Hirata et al. (1978) after isolation of the phospholipids by thin-layer chromatography.

Results

The systolic blood pressure of SH and WKY rats at 14 weeks of age was 180 ± 10 and 110 ± 15 mmHg respectively.

In the pineal of SH rats, the dopamine levels increased 34%, the adrenaline levels increased 152% and the noradrenaline concentration decreased 26% with respect to the concentrations in normotensive controls. In hypertensive rats, adrenaline represents 13% of the total catecholamines in the pineal gland (Table I), where it constitutes only 4% of total catecholamines in normotensive rats.

The activity of pineal N-acetyltransferase, measured at 10.00 hours, was very small in normotensive animals. In hypertensive rats the activity was four times higher than in controls (Table I).

The incorporation of [³H]methyl groups from [³H]methyl-S-adenosylmethionine into specific phospholipids was increased in hypertensive rats (140%, 320% and 100% for phosphatidylmonomethyllethanolamine, phosphatidyldimethylethanolamine and phosphatidylcholine respectively) compared with that in normotensive controls (Table I).

Discussion

In spontaneously hypertensive rats, stress results in higher blood levels of adrenaline than in normotensive controls (McCarty & Kopin, 1978). The results suggest that spontaneously hypertensive rats can take up more adrenaline from the circulation into the pineal sympathetic nerves, or, alternatively, that the pineal glands of hypertensive animals possess a higher capacity for production of adrenaline. In addition, a decreased concentration of noradrenaline, in the presence of increased dopamine concentration, is in favour of an enhanced release of noradrenaline from pineal sympathetic nerves in hypertensive animals.

The increased noradrenaline release could be due to the uptake of adrenaline by the pineal sympathetic nerves and displacement of noradrenaline to the synaptic cleft, or it could be...
due to an action of adrenaline on presynaptic stimulatory β-receptors (Rand, Majewski, McCulloch & Story, 1979).

The enhanced stimulation of pineal β-receptors in hypertensive rats, as evidenced by increased N-acetyltransferase activity, could be related to the increased release of noradrenaline, and also to a direct effect of adrenaline in the postsynaptic β-receptors of the pinealocytes. Adrenaline, at a concentration similar to that reported here, has been shown to produce substantial postsynaptic β-receptor stimulation in vitro (Parfitt & Klein, 1976).

The increased stimulation of pineal β-receptors by adrenaline, noradrenaline or a combination of both suggests that the pineal of SH rats could produce more melatonin than the corresponding normotensive controls.

Our results support the hypothesis (Rand et al., 1979) that hypertension may present a change in the transmitter released from peripheral sympathetic nerves, such that it includes a proportion of adrenaline. If increased adrenaline concentrations in peripheral sympathetic nerves results in increased noradrenaline release by presynaptic β-receptor stimulation, the anti-hypertensive effects of the adrenoceptor blockers could be partially explained as a blockade of these presynaptic receptors.

Membrane phospholipid methylation is increased in pineal gland of hypertensive rats. Increased phospholipid methylation may also occur in other sympathetically innervated tissues in hypertensive subjects. If this would be the case, and since methylation of membrane phospholipids could affect the coupling of the β-receptors to the adenylate cyclase, it is tempting to speculate that inhibitors of phospholipid methylation could affect the response of β-receptors in hypertensive subjects. Such drugs could become important tools for the investigation and treatment of hypertension.

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References


