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Effect of hypo- and hyperthyroidism on binding of [^{3}H]-nitrendipine to myocardial and brain membranes

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The density of calcium channel binding sites as determined by [^{3}H]-nitrendipine binding was found to be decreased in hearts of hyperthyroid rats but hardly altered by hypothyroidism. In contrast, dihydropyridine binding sites in the cerebral cortex were unaffected by dysthyroid states. Myocardial [^{3}H]-nitrendipine binding sites were also decreased after treatment of the animals with isoprenaline but not in spontaneously hypertensive rats. These findings suggest that myocardial hypertrophy is not necessarily accompanied by a loss of calcium channels and that thyroid hormone regulates the density of [^{3}H]-nitrendipine binding sites in a tissue-specific manner.

Keywords [^{3}H]-nitrendipine binding Ca^{++}-channels thyroid hormone myocardial hypertrophy spontaneously hypertensive rats

Introduction

Dysthyroid states induce a variety of changes in the heart, e.g. hypo- or hypertrophy, changes of adrenergic mechanisms and altered myocardial contractility. Hyperthyroidism, for instance, increases the number of β-adrenoceptors, decreases the number of α_1-adrenoceptors (Gross & Lues, 1985) but impairs the positive inotropic response mediated by α_1- as well as β-adrenoceptors in right ventricles of rats (Gross & Hanft, unpublished results). Since an effect of thyroid hormone on calcium channels may contribute to altered cardiac contractility we investigated whether hypo- and hyperthyroidism affect dihydropyridine binding sites associated with calcium channels. Changes induced by hyperthyroidism were compared with alterations caused by other treatments or diseases which cause myocardial hypertrophy.

Methods

Male adult Wistar rats (initially weighing 200 to 300 g) or Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR, 22 weeks old) were used for the experiments. Hypothyroidism was induced by giving 0.05% 6-propyl-2-thiouracil (PTU) with the drinking water for at least 3 weeks, hyperthyroidism by daily injections of L-triiodothyronine (T_3; 500 μg kg^{-1} i.p., 7 days). Isoprenaline (ISO) was injected to induce myocardial hypertrophy (3 mg kg^{-1} twice daily for 14 days). The positive inotropic response in right ventricles was determined as described previously (Gross *et al.*, 1988).

Dihydropyridine binding sites in crude membrane fractions of cerebral cortex and heart ventricles were labelled by [^{3}H]-nitrendipine (12 concentrations ranging from 0.05 to 1.6 nM; specific activity 70–74 Ci mmol^{-1}, NEN, Boston, USA) as described by Glossmann & Ferry (1985). Membranes were incubated at 21° C for 90 min in a final volume of 1 ml (incubation buffer: Tris HCl 50 mM, pH 7.4). Non-specific binding was defined by 100 nM nitrendipine and amounted to 20 to 30% of total binding at 0.3 nM [^{3}H]-nitrendipine. Means ± s.e. mean are given; means were compared by ANOVA and Duncan's multiple range test.

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Results

PTU-induced hypothyroidism and T_3-induced hyperthyroidism caused cardiac hypo- and hypertrophy, respectively. In right ventricles of euthyroid rats the maximum increase in contractile force elicited by raising the extracellular Ca^{++} concentration from 1.2 to 7.2 mM amounted to 0.75 ± 0.04 mN mg^{-1} dry weight, n = 8. Pretreatment of the animals with PTU did not significantly change this response (0.91 ± 0.08 mN, n = 8), whereas daily injection with T_3 significantly ($P < 0.01$) attenuated this response by 52% (0.36 ± 0.05 mN mg^{-1} dry weight, n = 8).

[^{3}H]-nitrendipine bound to heart and brain membranes with mean pK_D values of 9.3 to 9.9 (−log M). Pretreatment with PTU, T3 and isoprenaline and spontaneous hypertension did not influence the affinity of the radioligand to its binding sites (Table 1). Likewise, the inhibition of [^{3}H]-nitrendipine binding (0.3 nM) by PN 200–110, Bay K 8644 and verapamil was not altered in these groups (data not shown). PTU-induced hypothyroidism decreased the number of [^{3}H]-nitrendipine binding sites per heart. However, B_{max} values referred to tissue wet weight or protein content of the membranes remained unaltered or increased slightly. In contrast, T_3 treatment significantly reduced the absolute number as well as the concentration of binding sites. Cardiac hypertrophy due to isoprenaline but not the hypertrophy in spontaneously hypertensive rats was accompanied by a significant decrease in the density of [^{3}H]-nitrendipine binding sites (Table 1).

Discussion

Our experimental data show that thyroid hormone alters the density of [^{3}H]-nitrendipine binding sites in rat heart but not in cerebral cortex. PTU treatment decreased heart weight and the amount of dihydropyridine binding sites per heart to about the same extent resulting in an unaltered or slightly increased concentration referred to tissue weight or protein content of the membranes, respectively. In contrast, T_3 treatment decreased the number of [^{3}H]-nitrendipine binding sites per heart although ventricular mass was enhanced. These findings partly confirm a previous report of Hawthorn *et al.* (1988) who found a significantly increased density of dihydropyridine binding sites in hypo- and a decrease in hyperthyroidism. The interpretation of their results, however, is complicated by the fact that myocardial hypo- and hypertrophy was not taken into account. Therefore, it remained unclear whether the observed effects might be explained simply by concentration and dilution of calcium channels due to an increase and decrease in ventricular mass, respectively, or may reflect a real alteration of the synthesis and/or degradation induced by thyroid hormone. Our results suggest that T_3 treatment indeed regulates the number of calcium channel associated dihydropyridine binding sites. This effect obviously does not occur in all organs. Such tissue specific influences of thyroid hormone are well known for other binding sites, e.g. adrenergic receptors. Finally, an indirect effect due to hypertrophy of the heart and independent of thyroid hormone remained to be ruled out.

Table 1 [^{3}H]-nitrendipine binding to membranes of rat heart ventricles (a) and cerebral cortex (b). Effect of PTU-induced hypothyroidism, T_3-induced hyperthyroidism, isoprenaline pretreatment and spontaneous hypertension

Tissue	*Treatment*	K_D *(−log M)*	B_{max} *(fmol mg^{-1} protein)*	B_{max} *(fmol mg^{-1} wet weight)*	*(pmol heart)*	*Tissue wet weight (mg)*	
a. *Heart*	Control	9.6 ± 0.1	150 ± 4	16 ± 1	17 ± 1	1088 ± 48	9
	PTU	9.6 ± 0.1	163 ± 4*	16 ± 1	12 ± 1**	754 ± 22**	9
	T_3	9.6 ± 0.1	99 ± 4**	11 ± 1**	14 ± 1**	1286 ± 40**	9
	WKY	9.3 ± 0.1	208 ± 10	21 ± 1	20 ± 1	961 ± 25	6
	SHR	9.3 ± 0.1	189 ± 9	21 ± 1	27 ± 2**	1242 ± 29**	6
	Control	9.9 ± 0.1	225 ± 6	18 ± 1	16 ± 1	903 ± 7	6
	ISO	9.9 ± 0.1	174 ± 9**	12 ± 1**	15 ± 1	1223 ± 18**	6
b. *Cerebral cortex*	Control	9.5 ± 0.1	144 ± 9				9
	PTU	9.4 ± 0.1	138 ± 7				9
	T_3	9.5 ± 0.1	146 ± 8				9

* $P < 0.05$, ** $P < 0.01$ (Duncan's multiple range test). Means ± s.e. mean.

Therefore, we studied possible alterations of [^{3}H]-nitrendipine binding sites in other kinds of myocardial hypertrophy. Like T_3 treatment isoprenaline decreased the density of binding sites. In spontaneously hypertensive rats, however, hypertrophy of the myocardium was not accompanied by a reduction of dihydropyridine binding sites. These results are consistent with the results of Ishii *et al.* (1983, 1988) but disagree with a previous report of Chatelain *et al.* (1984). Myocardial hypertrophy induced by pressure overload has also been reported not to affect the concentration of calcium channels (Mayoux *et al.*, 1988) supporting our view that hypertrophy of the heart is not necessarily accompanied by a loss of dihydropyridine binding sites and that T_3 may directly regulate the density of calcium channels. The functional relevance of the T_3-induced decrease in calcium channels remains to be investigated further. It may contribute to the impaired contractility of the heart due to elevation of extracellular Ca^{++} (see above) and calcium channel activators (own unpublished results).

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