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Short communication

Central α_2-adrenoceptors and blood pressure regulation in the rat

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Abstract

The role of α_2-adrenoceptors in the central regulation of blood pressure has been questioned, since drugs such as clonidine stimulate both α_2-adrenoceptors and imidazoline-preferring receptors. The present work was undertaken to study the influence of α_{2D}-adrenoceptors, encoded by the RG20 gene, on blood pressure in the rat. An antisense phosphodiester oligodeoxynucleotide, directed at nucleotides 4–21 of the RG20 gene, was injected in the right lateral cerebral ventricle, causing an increase in systolic blood pressure, both at 1 and 2 days after the injection, when compared to groups of control rats (injected with the sense oligodeoxynucleotide or a missense oligodeoxynucleotide or distilled water). Antisense oligodeoxynucleotides directed either at nucleotides 65–82 of the RG10 gene (encoding for α_{2C}-adrenoceptors) or at nucleotides 26–43 of the RNG gene (encoding for α_{2B}-adrenoceptors), failed to produce any change in blood pressure after being injected. We conclude that the α_2-adrenoceptor encoded by the RG20 gene may play a role in blood pressure regulation in the rat.

Keywords: α-Adrenoceptor; Blood pressure; Central nervous system; RG20 gene; (Rat)

1. Introduction

While adrenaline or noradrenaline raise blood pressure when acting systemically, they produce a decline in blood pressure when delivered in the cerebrospinal fluid (Marley and Stephenson, 1972). On the other hand, clonidine and methyldopa show a blood pressure-lowering effect when acting systemically, thereby being currently used as antihypertensive drugs. It has been postulated that clonidine lowers blood pressure by stimulating α-adrenoceptors in the central nervous system (for review see Schmitt, 1977; Kobinger, 1986; McGrath et al., 1989). However, recent evidence has shown that the blood pressure-lowering effect of clonidine may be due to the stimulation of receptors other than α-adrenoceptors (imidazoline-preferring receptors) (Bousquet et al., 1984). In the rat, the α_2-adrenoceptor subtype which predominates in the brainstem has been shown to correspond to the receptor encoded by the RG20 gene (Zeng and Lynch, 1991; Lanier et al., 1991). The α_2-adrenoceptor encoded by the RG20 gene has been classified as the α_{2D} subtype (Lanier et al., 1991); the α_{2D} subtype might be the rat homolog of the human α_{2A} subtype (MacKinnon et al., 1994). In order to check whether α_2-adrenoceptors are involved in blood pressure regulation in the rat, we studied the effect on blood pressure of the intracerebroventricular injection of antisense oligodeoxynucleotides complementary to segments of three genes: the RG20 gene (encoding for the α_{2D}-adrenoceptor; Lanier et al., 1991), the RG10 gene (encoding for α_{2C}-adrenoceptors; Lanier et al., 1991) and the RNG gene (encoding for α_{2B}-adrenoceptors; Zeng et al., 1990).

2. Materials and methods

Male Wistar rats, weighing 270–310 g at the moment of the intracerebroventricular injection, were used. Oligodeoxynucleotides (phosphodiester) were stereotactically injected into the right lateral cerebral ventricle (David Kopf Instrument, Tujunga, CA, USA), with a single injection per rat (a volume of 20 μl, corresponding to 4 nmol, was injected), under general anaesthesia induced with pentobarbital sodium (50 mg/kg). The oligodeoxynucleotides (18-mers) were obtained from Pharmacia Biotech Benelux (Roosendal, Netherlands) and had the following composition: RG20ASP (antisense, directed at nucleotides 4–21 of the RG20 gene) – 5'ATCCGGCTGCAGGGAGCC 3'; RG20SP – (sense; nucleotides 4–21 of the RG20 gene) 5' GGCTCCCTGCAGCCGGAT 3' (Lanier et al.,

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Table 1
Blood pressure (systolic and diastolic; mm Hg) of rats injected with either distilled water or an oligodeoxynucleotide (see text for composition), 1 and 2 days after the intracerebroventricular injections (day 1 and day 2, respectively)

	Day 1	Day 2
Water	132.6±2.1/73.9±2.2	124.3±1.9/77.0±4.0
RG20ASP	147.0±3.1 [a]/78.5±4.0	146.9±4.0 [a]/75.9±4.3
RG20SP	127.3±2.8/72.8±5.4	124.1±2.1/68.0±2.6
RG20MSP	128.4±4.8/68.8±3.4	129.2±4.7/71.1±7.6
RG10ASP	129.9±3.3/69.7±2.4	126.2±3.5/75.0±3.5
RNGASP	132.6±3.5/69.4±4.2	127.5±4.2/68.0±2.5

[a] $P < 0.05$ vs. all other groups.

1991); RG20MSP – (missense; mismatches from RG20ASP underlined) 5′ ATCCAGCGGCTGGGAGCC 3′; RG10ASP – (antisense, directed at nucleotides 65–82 of the RG10 gene) 5′ TACCCCATTCGCCCGCGT 3′; RNGASP – (antisense, directed at nucleotides 26–43 of the RNG gene) 5′ GCGCTCCGAAAAGGCGCT 3′. The sequences of the oligonucleotides used were checked against all other rat sequences present in the EMBL data library (European Molecular Biology Laboratory, Heidelberg, Germany), and were chosen in such a way as to avoid very high G + C contents (Wahlestedt, 1994). These experiments were carried out under aseptic conditions.

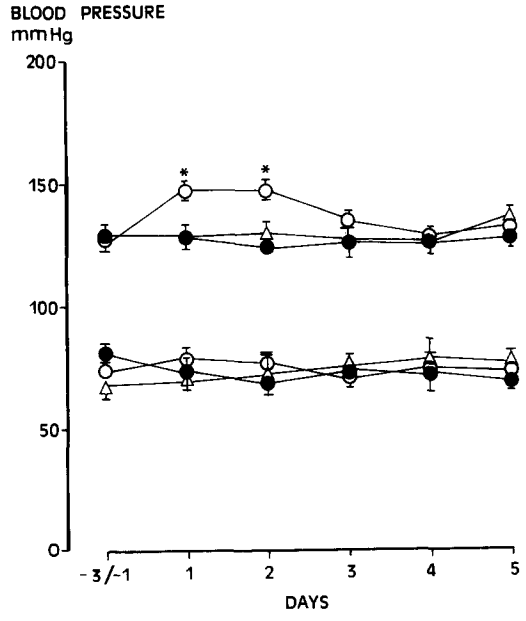

Fig. 1. Blood pressure (systolic and diastolic; mm Hg), measured in the rat tail. Open circles: rats injected with antisense oligodeoxynucleotide, directed at nucleotides 4–21 of the RG20 gene; closed circles: rats injected with sense oligodeoxynucleotide; open triangles: rats injected with missense oligodeoxynucleotide. Days −3/−1: average of blood pressure values in the 3 days before the injections had been performed. Days 1–5: days after the injections had been performed. * $P < 0.05$ vs. corresponding values in rats injected with either sense or missense oligodeoxynucleotides.

The blood pressure was measured by means of the tail-cuff method, using a Letica digital pressure meter LE 5000 (Letica, Barcelona, Spain). The rats were pre-warmed to 38° C, in plastic cages, five measurements of blood pressure were obtained, and the arithmetic means were calculated. Blood pressure measurements were recorded for 4 days before the intracerebroventricular injections, and for 5 days after.

The results are presented as arithmetic means ± S.E.M. Student's t-test was used to compare pairs of means, and a P level of 0.05 or less was considered significant.

3. Results

When compared to the control rats, the rats injected with RG20ASP ($n = 9$) showed a significant increase in systolic blood pressure at days 1 and 2 after the injections (Table 1; Fig. 1). The rats injected with distilled water ($n = 9$), RG20SP ($n = 6$), RG20MSP ($n = 5$), RG10ASP ($n = 6$) or RNGASP ($n = 6$), showed similar values of blood pressure. In days 3–5 after the injections, the different groups of rats showed similar values for systolic blood pressure. Regarding diastolic blood pressure, there was no significant difference when the different groups of rats corresponding to different treatments were compared.

4. Discussion

The present work was undertaken to study the role of central α_2-adrenoceptors in blood pressure regulation in the rat. An antisense oligodeoxynucleotide, directed at nucleotides 4–21 of the RG20 gene (Lanier et al., 1991), was injected in the right lateral cerebral ventricle and an increase in systolic blood pressure was observed on the first and second days after the injection. The observed increase in blood pressure may have been due to an interaction between the injected oligodeoxynucleotide and the messenger RNA corresponding to the RG20 gene, leading to α_2-adrenoceptor down-regulation in the central nervous system. Direct evidence to test this mechanism, however, was not obtained. If one assumes that the observed effect was in fact due to α_2-adrenoceptor down-regulation in the central nervous system, then one has to conclude that a rapid turnover of α_{2D}-adrenoceptors may exist in central nervous system regions involved in blood pressure regulation. The effect observed after the injection of RG20ASP lasted for 2 days, which may correspond to the clearance rate of the oligodeoxynucleotide by the rat central nervous system.

In the present experiments only systolic blood pres-

sure was found to change significantly; this may have been due to a greater accuracy of the tail-cuff method in measuring systolic than diastolic blood pressure. Considerable evidence indicates that α_2-adrenoceptors are involved in the regulation of blood pressure. This evidence is based above all on the fact that drugs such as clonidine which act as agonists on α_2-adrenoceptors, lower blood pressure (for review see Schmitt, 1977; Kobinger, 1986; McGrath et al., 1989). However, recent evidence challenged this concept, since it was found that clonidine and clonidine-like drugs seem to bind both to α_2-adrenoceptors and to a distinct type of receptors, imidazoline-preferring receptors (Bousquet et al., 1984). Zeng and Lynch (1991) and Nicholas et al. (1993) showed a differential distribution of mRNAs corresponding to the RG20, RG10 and RNG α_2-adrenoceptor genes in the rat brain. In the brainstem, the RG20 gene seems to predominate (Zeng and Lynch, 1991); this is in good agreement with the present results, since only the antisense oligodeoxynucleotide corresponding to the RG20 gene produced an effect on blood pressure, whereas the antisense oligodeoxynucleotides directed at either the RG10 gene or the RNG gene failed to produce any effect on blood pressure. These negative results, however, do not prove that the RG10 and the RNG genes are not involved in blood pressure regulation in the rat. α_2-Adrenoceptors have been subclassified, based mainly on radioligand binding data, into four different subtypes (A, B, C and D subtypes) (MacKinnon et al., 1994). The α_{2A} subtype appears to be non-existent in the rat, and it has been suggested that the α_{2D} subtype is the species homolog in the rat of the human α_{2A}-adrenoceptor subtype (MacKinnon et al., 1994). Oligodeoxynucleotides have a short half-life in plasma, due to the presence of exonucleases (Crooke, 1992); however, in the cerebrospinal fluid, phosphodiester oligodeoxynucleotides seem to be relatively stable (Wahlestedt, 1994). Although multiple intracerebroventricular injections of phosphodiester oligodeoxynucleotides have been used to produce pharmacological effects (Wahlestedt et al., 1993), a single injection was used in the present study. The single injection may decrease the potential for contamination of the cerebrospinal fluid by plasma nucleases. We conclude that the α_2-adrenoceptor encoded by the RG20 gene may play a role in blood pressure regulation in the rat.

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