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κ-Opioid receptor antisense oligonucleotide injected into rat hippocampus causes hypertension

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Abstract

Bi-hippocampal microinjection treatment (1 μg per side, twice a day for 5 days) with an antisense phosphorothioate oligodeoxynucleotide antisense oligodeoxynucleotide to the rat κ-opioid receptor, caused hypertension in normotensive Wistar Kyoto (WKY) rats and increased the blood pressure of spontaneously hypertensive rats (SHR). Systolic blood pressure in WKY rats increased from 121 ± 4 to 153 ± 6 mm Hg, and in SHR systolic blood pressure increased from 153 ± 4 to 183 ± 5 mm Hg. Similar results were observed with mean blood pressure, however, there were no changes in heart rate. No significant responses were seen with either vehicle or missense injections. Radioligand binding studies indicated that there was a significant decrease in apparent κ-opioid receptor density due to antisense oligodeoxynucleotide treatment. The results are in accord with our earlier suggestions that the κ-opioid system in the hippocampus may have a role in the neural control of blood pressure.

Keywords: κ-Opioid receptor; Hippocampus; Blood pressure; Antisense oligonucleotide

1. Introduction

Recent advancements in molecular pharmacology have equipped the scientist with a new, selective tool — antisense technology (Pasternak and Standifer, 1994). Antisense oligonucleotides (AS Oligos) have been successfully used in many studies to reduce receptor density and function (Wahlestedt, 1994) and are being explored therapeutically (Matteucci and Wagner, 1996; Nyce and Metzger, 1997). AS Oligos are believed to act by either binding to mRNA or directly to the target gene. This process may prevent the transcription to mRNA or the translation of the mRNA into protein. The precise mechanism concerning the action of AS Oligos remains controversial; however, two mechanisms have been proposed (Helene, 1991; Tonkinson and Stein, 1996). The first mechanism involves RNase disruption of the hybrid and therefore inhibition of chain elongation. The second proposes that AS Oligos prevent the reading of the template by blockade of translational factors. AS Oligos targeting the κ-opioid receptor have been shown to selectively inhibit κ-agonist analgesia in rats (Adams et al., 1994) and mice (Chien et al., 1994). This is the first report of using AS Oligos to target the κ-opioid receptor in hypertension.

Previous research in this laboratory established that the dynorphin-A (1–8) level in the hippocampal formation is 75% lower in spontaneously hypertensive rats (SHR) than in normotensive Wistar Kyoto (WKY) controls (Li et al., 1989). It was also shown that injections of intrahippocampal dynorphin-A (1–8) caused dose-dependent hypotension and bradycardia in all strains and ages of rats, via a mechanism which appeared to involve κ-opioid receptor activation in the hippocampus (Wang and Ingenito, 1994). This effect was observed in both anesthetized and conscious rats. The hippocampal area appeared to be tonically inactive because inactivation with lidocaine did not lead to any cardiovascular changes. These findings suggested that dynorphins, acting via hippocampal κ-opioid receptors, may function to restrain blood pressure elevations, and that the relative decrease in dynorphins or κ-opioid receptors may lead to hypertension. An increase in κ-opioid receptor density has been found in the SHR brain (Bhargava and Das, 1986) possibly as an up-regulation in response to low dynorphin levels in SHR brain. The AS Oligo used in this study, designed by Adams, has previously been shown to have efficacy in inhibiting κ-mediated analgesia in rats (Adams et al., 1994). It was the purpose of this study to

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determine if administration of an AS Oligo directed to the κ-opioid receptor would lead to increased hypertension in SHR and provoke hypertension in normotensive WKY controls.

2. Materials and methods

2.1. Experimental animals

The investigation was performed with male SHR and WKY rats, at 10 weeks of age, purchased from Harlan Laboratories. At this age hypertension is just beginning to develop in SHR and reaches its maximum at about 16 weeks of age. Animals were housed three or four to a cage in our animal facility and were maintained on a 12/12 h light cycle in a controlled environment at constant temperature of 23°C and humidity of 50 ± 10%. Animals were fed standard rat chow and water ad libitum. Each animal was only used once.

2.2. Physiological measurements

Systolic and mean arterial blood pressure and heart rate were determined in rat tail arteries with an IITC model 31, computerized tail cuff apparatus. The procedure required a minimum of a 3-day training period, to allow the animals to become acquainted with the brief immobilization in a Plexiglas holder (maximum of 20 min) and a slight elevation of temperature to 28°C in an environmental chamber. Three replicate measurements were taken from each animal daily at the same time of the morning. The average of three determinations of systolic and mean arterial pressure and heart rate were recorded. Prior to drug administration, blood pressure measurements were recorded daily for 6 continuous days. The before treatment values reported here were the means of 6 days pre-drug. After drug administration, measurements were taken daily until an effect was seen on day 3 of AS Oligo administration. Blood pressure measurements were then recorded twice daily until the experiment was terminated on day 6. The after treatment values reported here were the means of the blood pressure determinations from days 4 and 5.

2.3. Intra hippocampal microinjection of AS Oligo

Rats were anesthetized with methohexital sodium (Brevital, 70 mg/kg, i.p.) and stereotaxically implanted with permanent bilateral stainless steel guide cannulas (22-gauge, length = 11 mm) in the hippocampus (from bregma; anterior/posterior (AP) = −4.0 mm, medial/lateral (ML) = ±1.6 mm, dorsal/ventral (DV) = −3.0 mm, in the flat head position). We previously established that these sites elicit blood pressure lowering responses to injections of dynorphins and non-peptide κ-opioid receptor agonists (Wang and Ingenito, 1994). After the surgery the animals were allowed a recovery period of 5 days.

Rat κ-opioid receptor phosphorothioate AS Oligo was synthesized according to Adams et al. (1994). Each animal received 1 μg in 0.5 μl, sterile water for injection, per side twice a day (via a 30-gauge injection needle attached by PE-10 tubing to a 1 μl Hamilton syringe) containing either antisense (5′-AAT CTG GAT GGG GGA CTC-3′), missense (5′-GAC TGG AGG CAC TTG ATG-3′) or only sterile water for injection (vehicle). The depth of the hippocampal injections was 3.1–3.2 mm, and the injector end extended 1 mm beyond the guide cannula. Animals were injected at 8 a.m. and 8 p.m. every day for 5 days and blood pressures were determined each day beginning at 9 a.m. Little pharmacokinetic data are available concerning AS Oligos. However, the plasma half-life of unmodified AS Oligos following intravenous administration is generally less than 5 min (Agrawal et al., 1995). Modified AS Oligos have plasma half-lives ranging from minutes to hours, depending upon the dose, site of administration,

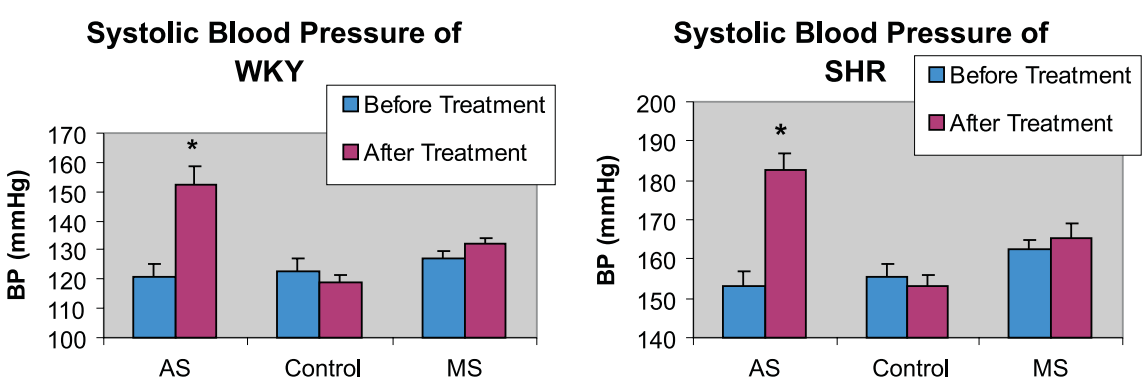

Fig. 1. Blood pressure (BP) measurements of WKY (a) and SHR rats (b) treated with either AS Oligo (AS), missense (MS) or vehicle (control). Bihippocampal microinjection of 1 μg/0.5 μl per side, twice a day for 5 days. After treatment values are means of blood pressure determinations from days 4 and 5 days during microinjection treatment. $^{*}P < 0.0001$, when compared with values before drug administration. The number of animals used for each group are as follows; AS Oligo treated WKY rats, $n = 9$, AS Oligo treated SHR, $n = 10$, both SHR and WKY control groups, $n = 6$ and both SHR and WKY MS control groups, $n = 8$.

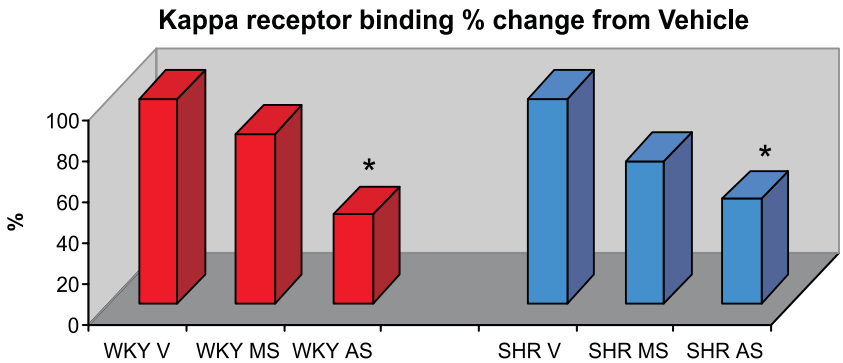

Fig. 2. Kappa receptor binding of WKY and SHR rats treated with either AS Oligo (AS), missense (MS) or vehicle (V). Each value represents mean values ($n = 8–14$). $^{*}P < 0.05$, according to Student–Newman–Keuls ANOVA multiple comparisons.

type of modification and animal used (Akhtar and Agrawal, 1997). The dose used was calculated by considering the dose that Adams et al. (1994) used and then considering how other researchers have used AS Oligos in specific brain nuclei. These doses should be less than those injected into the cerebral ventricles. Receptor turnover of opioid receptors is thought to be 2–3 days (Pasternak et al., 1980). Animals were euthanized 12 h after the final injection with sodium pentobarbital (60 mg/kg i.p.) and decapitated. The brains were rapidly removed and the dorsal hippocampus was dissected and stored at −70°C until radiobinding analysis could be completed. Actual injection sites into the dorsal hippocampus were confirmed by histological examination on three control animals. In the experimental animals, examination of the exact injection sites were confirmed during hippocampal dissection.

2.4. Binding analysis

The κ-opioid receptor binding in rat hippocampal membranes was determined according to our previously established protocol using 1.2 nM [^{3}H]-bremazocine to label the κ-opioid receptors in the presence of 100 nM DAMGO (D-ala^{2}, (Me)phe^{4}, glyol^{5}) and DPDPE (D-penicillamine^{2}, D-penicillamine^{5}-enkephalin) to block the μ and δ receptors, respectively (McConnaughey et al., 1998). Non-specific binding was determined in the presence of 1 μM non-radioactive bremazocine and protein determination was performed by the method of Lowry et al. (1951).

Table 1
Radioligand binding of hippocampal κ-opioid receptors. Values expressed as mean ± S.E.M. V = vehicle, MS = missense and AS = AS Oligo

Group	pmoles/mg protein	*n*
WKY V	0.068 ± 0.01	8
WKY MS	0.057 ± 0.003	14
WKY AS	0.030 ± 0.006[a]	10
SHR V	0.078 ± 0.02	10
SHR MS	0.055 ± 0.003	10
SHR AS	0.040 ± 0.005 [a]	14

[a] $P < 0.05$, Student–Newman–Keuls ANOVA multiple comparisons.

2.5. Statistics

The results are represented as means ± S.E.M. The systolic blood pressure data was analyzed by Student's paired *t*-test to determine the differences in measurements before and after administration of either AS Oligo, missense Oligo (MS) or vehicle. Radioligand binding results were analyzed using Student–Newman–Keuls ANOVA. Differences were considered to be statistically significant at $P < 0.05$.

3. Results

The results demonstrate that the AS Oligo caused a significant increase in systolic blood pressure in both SHR and WKY rats (Fig. 1a and b). The change in blood pressure caused by the AS Oligo was approximately the same in both rat strains. Similar results were observed with mean blood pressure, however, no changes occurred in heart rate. Mean arterial blood pressure and heart rate data are not shown. In addition, bihippocampal treatment with either missense or vehicle had no significant effect upon the blood pressure of either SHR or WKY rats (Fig. 1).

The radioligand binding data showed significant decreases in the apparent κ-opioid receptor density in the dorsal hippocampus (Fig. 2), of both SHR ($B_{max} = 0.039 \pm 0.005$) and WKY rats($B_{max} = 0.029 \pm 0.006$) treated with AS Oligo (Table 1).

4. Discussion

Opiates and endogenous opioid peptides are known to affect cardiovascular control (Feuerstein and Siren, 1987) by acting through opioid receptors in specific brain areas and nuclei that are known to control cardiovascular function (Calaresu et al., 1984). The hippocampus is not considered to be a cardiovascular regulatory center, however, it is an integral part of the limbic system (Papez, 1995), and emotion plays an important role in cardio-

vascular responses. In this report, we have shown that injection of κ-opioid receptor AS Oligo into the hippocampus can effectively increase blood pressure, thus confirming that the hippocampus is in fact involved in blood pressure control. By administering κ-opioid agonists or by manipulating κ-opioid receptors, we may have an alternative method of lowering blood pressure from drugs acting via more conventional mechanisms.

Dynorphin is the most prominent endogenous ligand for the κ-opioid receptor in the rat brain (Corbett et al., 1982). Colchicine injections into the dentate gyrus of the hippocampus have been found to destroy dynorphin-producing granule cells at this site (Privette et al., 1994). These cells are the sole source of prodynorphin derived opioid peptides to the hippocampus (McGinty et al., 1983). Colchicine treated SHR, have an accelerated hypertension, while in normotensive WKY control rats, the drug provokes hypertension (Privette et al., 1994). This suggests that dynorphins within the hippocampus may modulate the activity of the peripheral autonomic nervous system and that a dysfunction of this system within the hippocampus may contribute to the hypertensive state.

As previously mentioned SHR have significantly lower levels of hippocampal dynorphin-A (1–8) (Li et al., 1989) and hippocampal injection of this peptide causes hypotension and bradycardia (Wang and Ingenito, 1994). This hypotensive effect is greater in the SHR than the WKY (Wang and Ingenito, 1994), which could imply that the SHR κ-opioid receptors are upregulated due to lower expression of dynorphin rather than the SHR having a higher baseline level allowing further reduction of blood pressure than in the normotensive strains.

The results of the present study are consistent with the results of our previous studies showing that low dynorphin levels (Li et al., 1989; Privette et al., 1994; Wang and Ingenito, 1994) are related to higher arterial pressures in the SHR since our present data shows that κ-opioid receptor deficiency can lead to hypertension. An increase of greater than 20 mm Hg was seen in the systolic blood pressure of both SHR and WKY rats, after treatment with AS Oligo.

We had expected to find a greater increase in blood pressure in the AS Oligo treated SHR than the WKY rats because the lower hippocampal dynorphin in the SHR might make maximal κ-opioid receptor occupation critical for helping to maintain neural control of blood pressure. If receptors are reduced by antisense treatment, there would be both a deficiency of agonist and receptor in this strain. However, the increase in blood pressure, i.e., the absolute change, was about the same with the SHR and WKY groups. It is possible that we have observed a maximal change in both groups.

The missense has already been shown to be an adequate control containing equal numbers of like bases (Adams et al., 1994). In this study the missense injections in both SHR and WKY rats had no significant effect upon blood pressure or κ-opioid receptor density. The AS Oligo was designed to target the N-terminal region of the rat κ-opioid receptor that contains a G-string which appears to increase the effectiveness of antisense by increasing the affinity of binding to target sequences. This is the first report of using such an AS Oligo targeting the κ-opioid receptor in the hippocampus to affect blood pressure.

The κ-opioid receptor density in the dorsal hippocampus was significantly reduced in the AS Oligo treated groups of both strains of animals used as compared to missense and vehicle controls (Fig. 2). These data suggests that the AS Oligo used in this study increased blood pressure via specifically reducing κ-opioid receptor density in the hippocampus thus removing a neural restraining component of blood pressure regulation.

κ-opioid receptor agonists have been shown to have antihypertensive properties when infused subcutaneously over a 2-week period into spontaneous hypertensive rats (Zhai and Ingenito, 1997). However, the chronic therapeutic use of non-peptide κ-opioid agonists has not been attempted, possibly due to their potentially undesirable CNS side effects such as dysphoria and sedation (Peters and Gaylor, 1989). Numerous researchers have reported that opioid receptors consist of many subtypes with different selectivities for drugs. High affinity sites have been associated with the analgesic effects of narcotics (Pasternak et al., 1980). It is conceivable that the cardiovascular effects of opiates may involve low affinity binding sites, which may offer the possibility for drug selectivity and stereospecifity. It has been the hope of this laboratory that this may be true of the κ-opioid receptors, in particular. It is hoped that our findings may provide the incentive for a discovery of a more selective therapeutic antihypertensive, without the unwanted side effects presently associated with currently available κ-agonists.

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