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EARLY EXPOSURE TO NALOXONE INCREASES BLOOD PRESSURE IN NORMOTENSIVE AND HYPERTENSIVE RATS

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Continuous in utero and postpartum exposure of SH and WKY rats to naloxone results in a significant increase in their systolic blood pressure relative to respective control animals. After six weeks of age, however, naloxone was no longer effective in sustaining this increase in blood pressure. Chronic exposure to naloxone beginning at three weeks of age failed to produce any significant differences in blood pressure between treated and control animals. Although naloxone has been shown to elevate blood pressure in hypotensive states, this report represents the first example of an increase produced by the narcotic antagonist in the normotensive state.

Introduction

A considerable number of experimental observations have accumulated which suggest that endogenous opiates interact with the cardiovascular system. β-endorphin and various other opioid peptides have produced a prolonged lowering of blood pressure (b.p.) in laboratory animals (1-3). The narcotic antagonist naloxone blocked this effect, and was also effective in reversing shock induced hypotension (4-6). More recently naloxone was reported to prevent the decrease in blood pressure associated with sleep (7). No effect of naloxone on normal blood pressure has, however, been reported to date. Taken together, these and other studies (8-10) have established a link between the endogenous opiates and their receptors with blood pressure regulation. Recently we reported that brain opiate receptor concentrations are increased in adult spontaneously hypertensive (SH) rats, relative to their normotensive parent strain (WKY), and that this increase coincided with the appearance of elevated blood pressure in these animals (11). This correlation further implicated endogenous opiates in cardio-

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vascular control and suggested that perinatal intervention by naloxone may disrupt the endorphin system during a critical period and produce an increase in blood pressure at an earlier age. Previous studies have demonstrated that acute exposure to naloxone would not produce any measurable effects on blood pressure (2, 12) and therefore we elected to employ a sustained delivery system to evaluate the effects of the narcotic antagonist on blood pressure in SH and WKY rats. We have previously reported on a non-biodegradable polymer system containing naloxone which provides longlasting protection against the effect of narcotic agonists (13). This report indicates that continuous in utero and postpartum exposure of SH and WKY rats to naloxone results in a significant increase in their blood pressure relative to respective untreated control animals. Although naloxone has been shown to elevate blood pressure in hypotensive states, this report represents the first example of an increase produced by the narcotic antagonist in the normotensive state.

Materials and Methods

Male and female SH and WKY rats were obtained from Charles River Breeding Laboratories and maintained in our animal research colony. All animals were given unrestricted access to Purina Lab Chow and tap water. Estrus cycles were determined and the rats were mated on the afternoon of the proestrus day. On day 14 of pregnancy a non-biodegradable polymer in the form of wafers (ca. 1 cm^2) was implanted subcutaneously in the mid dorsal area of the back under light ether anesthesia. The polymer which weighed between 50-100 mg, provided for the sustained release of naloxone (1-2 µgm/hr) for a period of at least two weeks (13). One week postpartum the mothers were reimplanted with a new naloxone-polymer wafer to ensure that the pups would receive naloxone while nursing. To verify that the nursing pups obtained naloxone from their mothers' milk, a separate experiment was carried out in which radioactive naloxone was absorbed by the polymer wafer. Pregnant rats were implanted with the polymer as described above. Pups were sacrificed at one week intervals through three weeks after parturition. Blood samples and stomach contents were extracted, and analysis by radiochromatography and scintillation counting revealed that naloxone was present.

To eliminate the possibility that uterine differences would influence the results, pups were obtained from 3 different mothers per group each of which was implanted with a polymer while pregnant. Eighteen days after birth, male pups were implanted with the polymer and this procedure was repeated at two week intervals during the course of the experiment. Control mothers and pups were implanted similarly with polymer pieces that did not contain the drug. Urine samples were collected and analyzed periodically to ensure that naloxone was being released from the polymer. The animals did not suffer any observable ill effects from the drugs or its vehicle, and no significant difference in body weight between treated and control groups was observed. Rats were weaned on day 21 postpartum and

blood pressure measurements were begun on day 25. Rats were warmed for 10 min at 39°C prior to measurement of blood pressure. Systolic blood pressure and cardiac rate was determined at 3-4 day intervals in the morning in unanesthesized rats using tail cuff plethsmography (Narco Bio-systems). On day 25 the rats were too small to use standard tail cuffs to measure blood pressure accurately; therefore a modified occlusion (length 18 mm, diameter 10 mm) and sensor cuffs (length 10 mm, diameter 10 mm) were used. The accuracy of the indirect blood pressure tail cuff measurements was confirmed in some of the animals by direct femoral artery canulation. Blood pressure differences were maintained in all animals studied.

Results

At 25 days of age, systolic blood pressure of SH rats exposed to naloxone was significantly higher compared to the control SHR rats implanted with placebo polymer. Two-way analysis of variance indicates that the differences between the groups were significant at the level $P < .001$ ($F = 22.8$), (Fig. 1). The abnormally high blood pressure recorded at 25 days for the naloxone-SH rats was maintained during the course of the experiment. As the control SH animals matured and became hypertensive the difference in blood pressure between the two groups decreased gradually. After six weeks of age no significant difference between the naloxone treated and placebo SH rats was evident. A significant increase in systolic blood pressure was also recorded for the WKY rats receiving naloxone-polymer relative to their corresponding controls ($P < .001$, $F = 23.5$), (Fig. 1). The elevation in blood pressure was approximately 20 mmHg through six weeks of age and this difference could not be increased upon subsequent reimplantation of naloxone-polymer. Six weeks after birth, the naloxone treated animals no longer exhibited higher blood pressure values than the control group and differences were not reinstated through eight weeks of age. No differences in heart rate were observed for either SH and WKY rats when compared to placebo implanted controls.

Discussion

Our findings demonstrate that beginning with prenatal exposure, chronic naloxone can significantly alter blood pressure in both SH and WKY rats. The specific effect of the narcotic antagonist on the elevation of systolic blood pressure is different in the two strains and presumably is related

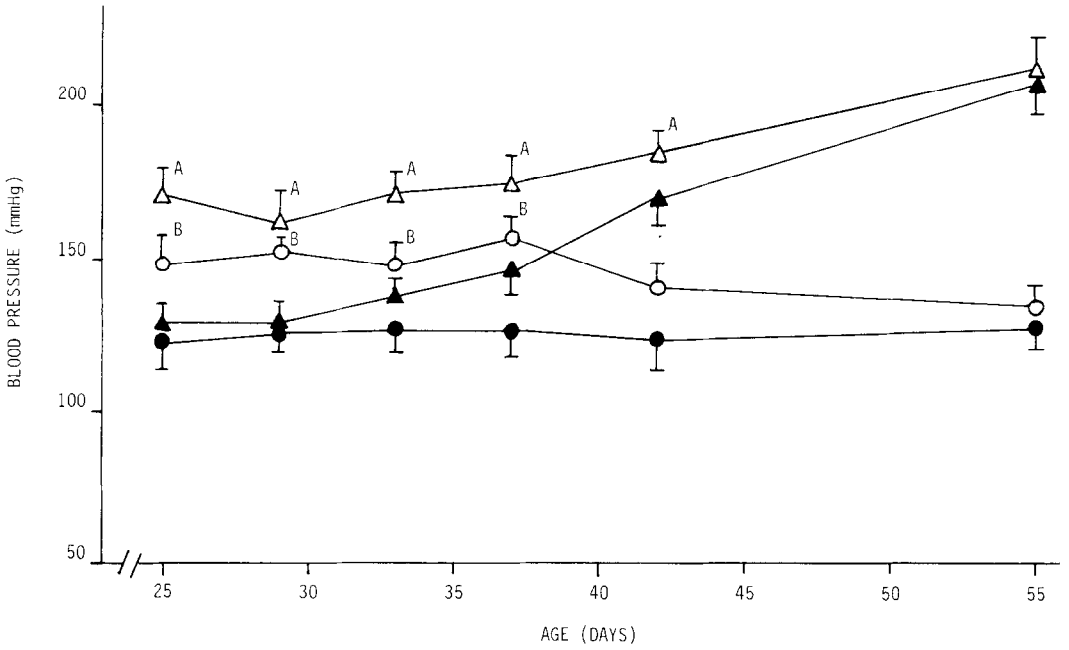

Fig. 1: Age and systolic blood pressure in spontaneously hypertensive (SH) and Wystar Kyoto rats (WKY). Each point represents the mean ± the standard error for 7 rats. SHR (▲—▲); SHR treated with naloxone (△—△); WKY (●—●); WKY treated with naloxone (o—o).
A - significantly different from SHR $P < .001$.
B - significantly different when compared to WKY $P < .001$.
Statistical comparisons between control and treated animals were performed using analysis of variance using the BMDp2v program developed at the Health Sciences Computing Facility, University of California, Los Angeles.

to the factors which contribute to the development of hypertension in the SH rat. Fig. 1 shows that the mean systolic blood pressure in the naloxone treated SH rat could not be raised above 225 mmHg. Thus the disappearance of blood pressure differences between the naloxone exposed and control SH rats is due to an increase in blood pressure in the latter animals with age. The inability of naloxone to increase blood pressure above a certain level suggests that a regulating mechanism may exist which limits the maximum blood pressure attainable in this hypertensive strain.

Blood pressure elevation relative to controls was also produced in WKY rats exposed chronically to naloxone (Fig. 1). However, in contrast to SH rats that were similarly treated, the blood pressure could not be raised above 150 mmHg. Furthermore, by six weeks of age naloxone was no longer effective in sustaining an increase in blood pressure since both control and naloxone treated animals exhibited similar systolic blood pressure. The

reason for the apparent development of tolerance to the effects of naloxone on blood pressure in this strain is not known and is currently under investigation. In both strains it is possible that naloxone loses its effect because of metabolic rather than pharmacodynamic reasons, since with increasing maturity the rat liver could cause a greatly increased rate of naloxone clearance. This possibility is being studied, but it should be noted that we have previously investigated similar polymer implants in mature CD rats and found that a single polymer wafer is effective in blocking the analgesic effects of morphine for 3-4 weeks (13).

To determine the effect of postnatal exposure to naloxone, polymer samples were implanted into three week old SH and WKT male rats. No significant difference in systolic blood pressure and heart rate was recorded between the naloxone treated animals and their respective controls through eight weeks of age (data not shown). Previous reports have suggested that early pharmacological intervention is more likely to produce physiological effects on the offspring of both normotensive and hypertensive strains of rats. Thus, prenatal naloxone exposure affects morphine sensitivity in rat offspring (14) while treatment of newborn SH rats with diazepam slows the development of high blood pressure in these animals (15). More recently, the absence of androgens during maturation of salt sensitive rats has been shown to sensitize the animals to hypertensive stimuli (16), and Tucker and Johnson (17) concluded from studies of sympathetic tone in neonatal SH rats that the first prenatal week may be a critical period during which interventions may be made to alter the sympathetic nervous system. Our results are consistent with these data that early drug exposure may have profound pharmacological consequences.

It is clear from the above results that in both SH and WKY rats naloxone produces an increase in blood pressure. The impact of the narcotic antagonist is not limited to animals genetically predisposed to hypertension but can also be seen in a normotensive strain. It is possible that this action may be indirect since naloxone has been shown to have

apparent agonist activity in behavioral tests and in in vitro assays and it has been reported to modify the effects of a number of non-opiate drugs (18, 19). In our study we have failed to observe any effects of naloxone on body weight, and recently other investigators have reported that early exposure to narcotic antagonists does not alter lactational behavior (14), reflex behavior and body growth (20) and spontaneous activity (21). Furthermore, the non-specific actions of naloxone require doses significantly higher than those required to produce classical opiate antagonist effects (19).

Our previous studies have implicated opiate receptors in the mechanisms controlling blood pressure (11). In conjunction with our present results the following hypothesis emerges. The effects of endogenous opiates on blood pressure are mediated by opiate receptors. In the SH rat a genetic defect leads to a disruption of endogenous opiate balance which is manifested by higher levels of opiate receptors and the appearance of elevated blood pressure as the animal matures. In the normotensive WKY rat, existing control mechanisms can minimize the impact of naloxone on blood pressure elevation of the narcotic antagonist.

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