Simultaneous Effect of Clonidine on Ambulatory Activity and Drinking Behavior in Spontaneously Hypertensive Rats

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The present experiment was undertaken to elucidate the simultaneous effect of clonidine on ambulatory activity and water drinking behavior in spontaneously hypertensive rats (SHR). An attempt was also made to determine the role of sympathetic nerve activity in the mechanism of clonidine-induced sedation in SHR. The sensitive method developed by Tadokoro et al. for simultaneous determination of ambulatory activity and drinking behavior in rats was used. Clonidine ($150 \mu\text{g}/\text{kg}$, po twice daily) produced a significant decrease in ambulatory activity and water drinking behavior at the beginning of the dark phase in twenty week old male SHR of Wistar Okamoto strain. Clonidine produced an increase in water drinking behavior in the light phase which was not observed in the control period. Under the same experimental conditions, the lowering of brain stem norepinephrine content produced by diethyl dithiocarbamate appeared to be inhibited in clonidine-treated SHR in the dark phase. Clonidine significantly decreased urinary norepinephrine content in the dark phase. On the other hand, clonidine ($30 \mu\text{g}/\text{kg}$, iv) produced a decrease in efferent nerve discharges of splanchnic nerve and sympathetic adrenal nerve in SHR. These findings suggest that decreased sympathetic nerve activity via a centrally mediated mechanism is at least in part associated with the decreased ambulatory activity induced by clonidine.

Key words: clonidine, ambulatory activity, drinking behavior, catecholamine

Adverse effects of clonidine on the central nervous system have been reported. The most frequent of these are sedation and dry mouth. McMahon¹⁾ calculated that the probability of clonidine induced sedation and dry mouth in patients with essential hypertension was more

than 5%. This clinical observation needs laboratory confirmation and explanation. Because of the close correlation with hypertension in humans, spontaneously hypertensive rats (SHR) is a good model of essential hypertension. With basic studies of clonidine in SHR, one can make reasonable predictions of adverse reactions in man²⁾. Although the sedative effect of clonidine was observed in animal experiments³⁾⁻⁶⁾, no re-

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ports are available in the literature concerning the simultaneous effect of clonidine on ambulatory activity and water drinking in SHR. Recently, Tadokoro et al.⁷⁾ developed a sensitive method for simultaneous determination of ambulatory activity and water drinking in rats. Using this new equipment, the present experiment was undertaken to elucidate the simultaneous effect of clonidine on ambulatory activity and water drinking in SHR. In order to clarify the mechanism of clonidine-induced sedative effect, an attempt was also made to determine the influence of clonidine on central and peripheral sympathetic nerve activity in SHR. Thus, the effects of clonidine on brain stem norepinephrine turnover, urinary catecholamine excretion and efferent discharge rate of splanchnic nerve and sympathetic adrenal nerve were examined in SHR.

Methods

Animals : Twenty week old male SHR of Wistar Okamoto strain (approximately 350 g) were used. The experiment was done in light-dark alternation cycle (light-dark cycle). The two phases were alternated every 12 hours. The light phase began at 8 : 00 a. m. and ended at 8 : 00 p. m.. Illumination was provided by fluorescent light (approximately 100 Lux). Room temperature was maintained at $20 \pm 2^\circ\text{C}$ throughout the experiment. Humidity was not controlled. Rats were fed a regular diet. Twice daily $150 \mu\text{g/kg}$ of clonidine was administered orally. First dose was given at 8 : 00 a. m. and second dose at 8 : 00 p. m., respectively. Water and drug were administered orally at a volume of 5 ml/kg. Studies on brain stem norepinephrine turnover and urinary catecholamine content were done after reversal of light-dark alternation cycle (dark-light cycle).

Measurement of blood pressure : The left carotid artery was cannulated for recording blood

pressure. The tubing (PE 20) was filled with 0.9% saline that contained 0.1% heparin and was run under the skin and out through the back of the neck. The end of the catheter was occluded with a needle. Patency of the catheter was maintained by flushing with 0.2 ml of heparinized saline twice daily. Carotid blood pressure was recorded with a pressure transducer (Nihon Kohden MUP-0.5 A) and a pen recorder. Two days after cannulation, $150 \mu\text{g/kg}$ of clonidine was administered in a single oral dose to each SHR. Before drug administration and every 3 hours thereafter, systolic and diastolic blood pressure was recorded.

Simultaneous determination of ambulatory activity and water drinking : The automatic Ambulo-Drinkometer (Ohara & Co. Ltd., Tokyo), developed by Tadokoro et al.⁷⁾, was used to determine ambulatory activity and water drinking in SHR. This apparatus is composed of ten steel rat cages [$39(\text{D}) \times 25(\text{W}) \times 17(\text{H})\text{cm}$], which are equipped with microswitches that are activated by tiltings of the cage floor as the rats move around. A water tank is connected to a drinking spout via an infusion machine, made of acryfiber and stainless steel tubes, which delivers 0.05 ml drops. The water falls drop by drop, as the rat drinks. There is a weak current in the upper part and lower part of the infusion machine. Short circuits generated by individual drops are amplified to activate an electromagnetic counter. Thus, using this apparatus, the exact amount and rate of water drinking can be determined⁸⁾. Nine rats were put into this apparatus 2 weeks prior to the study for adaptation. The ambulatory activity and water drinking behavior study was done in light-dark cycle. In the control period, water was administered twice daily for 5 days and in the drug treatment period, clonidine was administered for 10 days. Ambulatory activity and water

drinking were calculated as the mean of the total counts of the 9 rats during 3 hour periods. Ambulatory activity and water drinking in the drug administration period were compared with those in the control period (Fig.1).

Determination of brain stem norepinephrine turnover : The brain stem norepinephrine turnover study was done in dark-light cycle and was carried out on the third day of drug treatment period.

The dark phase in this study began at 8:00 a.m. and ended at 8:00 p.m. Norepinephrine turnover was calculated from changes in norepinephrine content in the brain stem after treatment with a depamine- β -hydroxylase inhibitor, diethyl dithiocarbamate (DDC)⁹. DDC at a dose of 500 mg/kg was given subcutaneously to the 2 groups 2 and 4 hours before sacrifice. On third day of the clonidine treatment period, rats were sacrificed in two groups, 3 and 12 hours after the

8:00 a.m. administration of clonidine (dark phase). Brain stem norepinephrine content in clonidine administered period was compared with the control which was given DDC alone. Brain stem norepinephrine content was measured by the trihydroxyindole method after separation of norepinephrine and epinephrine by high-performance liquid chromatography¹⁰ (Shimadzu LC-3 A).

Determination of urinary catecholamine content : Before drug administration and two days thereafter, urinary samples for catecholamine assay were collected every 3 hours during the dark phase and once during the light phase. Urinary catecholamine content was measured by the same method as used for the brain stem catecholamine content. It has been reported that urinary catecholamine excretion was related to the urinary creatinine concentration¹¹. For this reason, the value of catecholamine was expressed

Fig.1 Block diagram of the sensitive method for simultaneous determination of ambulatory activity and drinking behavior in rats.

in terms of ng/mg of creatinine. Creatinine was determined by the usual picric acid spectrophotometric method¹²⁾. At the same time, urine volume of each was measured.

Efferent discharge rate of splanchnic nerve and sympathetic adrenal nerve : Each SHR was anesthetized with urethane (1 g/kg, ip). Trachea was cannulated and animal was attached to artificial respirator (Princeton Medical Instruments, Inc. Model 131). Femoral vein was cannulated for drug administration. Intravenous injection was done at a volume of 0.2-0.3 ml and clonidine was administered at a dose of 30 μ g/kg. Splanchnic or adrenal nerve of either side was exposed retroperitoneally through flank incision. Splanchnic nerve or one adrenal nerve branch was dissected free from the surrounding tissues under binocular microscope. Efferent nerve discharges from the proximal cut end of splanchnic nerve or sympathetic adrenal nerve were recorded with bipolar platinum wire electrodes in warm liquid paraffin. Nerve activity was amplified and displayed on an oscilloscope (Iwatsu MS 5100 a) and polaroid photographs of tracings were taken. After passing through a window discriminator (W-P instruments model 120), the mean count of nerve activity per second was recorded continuously every ten seconds by a digital pulse counter (Hewlett-Packard 5300 A system) and displayed on a pen recorder. Statistical significance was evaluated by Student's t-test.

Results

Effect of clonidine on blood pressure in SHR : Single oral administration of clonidine at a dose of 150 μ g/kg produced a significant decrease in systolic blood pressure (approximately 30 mm Hg) 3 to 12 hours after drug administration. At the same time, a significant decrease in diastolic blood pressure was also observed (Fig. 2).

Effect of clonidine on ambulatory activity and water drinking in SHR : It was demonstrated that the 24 hour patterns of ambulatory activity and drinking behavior in SHR were well synchronized with the light-dark alternation cycle. The pattern of these two parameters remained nearly constant from day to day. Namely, the activity count of ambulation and drinking behavior in the dark phase was higher than that in the light phase. Furthermore, the ambulatory and drinking activity patterns showed two peaks in the dark phase. With water administration during the control period, ambulatory activity and water drinking decreased slightly, but activity patterns remained the same (Fig. 3).

Fig. 3 and Fig. 4 show the time course changes in mean ambulatory activity and mean water drinking in SHR during control period and clonidine-treated period over 24 hours. During the three hour period after the second dose of clonidine, a significant decrease in both ambulatory activity and water drinking as compared with the control period was demonstrated. On the contrary, the ambulatory activity during the three hour period before the first dose of clonidine was significantly elevated as compared with the control period. After the initial three hour period following the second dose of clonidine, the drinking counts surpassed those of the control. At the end of the light phase, a peak of drinking activity counts which was not observed in the control period was demonstrated (Fig. 4).

Effect of clonidine on brain stem norepinephrine turnover in SHR : In order to clarify whether clonidine influences brain stem norepinephrine turnover, an attempt was made to determine the influence of clonidine on the phenomenon of lowered brain stem norepinephrine brought about by administration of a dopamine- β -hydroxylase inhibitor, diethyl di-

Fig.2 Effect of clonidine on blood pressure in SHR. Effect was tested in two groups indicated by the following symbols: filled circles, age-matched control group; filled squares, clonidine administered group. Each value shows mean $\pm$ S.E.. Blood pressure was determined before and 3 hours, 6 hours, 9 hours and 12 hours after oral administration of clonidine. Statistical analysis was done by Student's t-test. Ordinate: systolic and diastolic blood pressure. Abscissa: time in hours.

thiocarbamate (DDC). In the brain stem norepinephrine turnover study, the light-dark alternation cycle was reversed (dark-light cycle): the dark phase began at 8:00 a.m. and ended at 8:00 p.m.. Ten days after alternating from light-dark cycle to dark-light cycle, SHR showed the same patterns of ambulatory activity and water drinking in the light phase of the light-dark cycle as in the light phase of the dark-light cycle. Similarly, behavior patterns of the dark phase of the light-dark cycle and the dark phase of the dark-light cycle showed identical rhythms. Namely, the activity counts of both dark phases were higher than those in the light phases, and showed two high peaks. At the beginning and the end of the dark phase, brain stem norepinephrine level was determined. When

control SHR were given a dose of 500 mg/kg sc of DDC, brain stem norepinephrine was lowered at both the beginning and the end of the dark phase. A lowering of brain stem norepinephrine produced by DDC appeared to be inhibited in the clonidine-treated SHR 3 and 12 hours after the 8:00 a.m. administration of clonidine in the dark phase. However, there was no significant difference between control and clonidine-treated SHR under these experimental conditions (Fig. 5).

Effect of clonidine on urine catecholamine content and urine volume in SHR: In the urinary study, the light-dark alternation cycle was also reversed to a dark-light cycle: the dark phase began at 8:00 a.m. and ended at 8:00 p.m.. Clonidine produced a significant increase in urine volume during the 3 hour period after the

Fig. 3 Effects of clonidine on ambulatory activity and drinking behavior in SHR. In the control period, water was administered twice daily for 5 days. In the drug administration period, clonidine was given twice daily for 10 days. Ambulation and drinking values were calculated as mean of total counts of 9 rats during 3 hour periods. Broken line indicates the ambulation activity during 24 hour period. Dotted line express water drinking activity during 24 hour period.

8:00 a.m. administration of clonidine (in the dark phase). During the following 3 hour period, clonidine produced a significant decrease in urine volume. Norepinephrine content in urinary samples which were collected 3 to 12 hours after the 8:00 a.m. administration of clonidine was decreased significantly as compared with samples taken during the control period. However, norepinephrine content of 12 hour urinary specimen in the light phase did not change. On the other hand, clonidine produced a statistically

insignificant decrease in urinary epinephrine content in the dark and light period (Fig. 6 and Fig. 7).

Effect of clonidine on efferent nerve discharges of splanchnic nerve and sympathetic adrenal nerve in SHR: Intravenous administration of clonidine at a dose of $30 \mu\text{g/kg}$ produced a significant decrease in efferent nerve discharges of splanchnic nerve and sympathetic adrenal nerve in SHR (Fig. 8). Intracerebroventricular administration of clonidine at a dose of $6 \mu\text{g}$

Fig. 4 Time course changes in rhythms of ambulation and drinking during control period and clonidine-treated period in SHR. In the control period, water was administered twice daily for 5 days. In the drug administration period, clonidine was administered twice daily for 10 days. In the control period, each value indicates the ambulation or drinking activity in 3 hour periods. Each value was calculated as mean of 5 day-summation of the 3 hour periods during pretreatment of 9 rats. Each value of ambulation and drinking activity of treatment period was expressed in terms of 3 hour periods. Each value was calculated as mean of 10 day-summation of 3 hour periods during treatment of 9 rats. Filled circles show age-matched control group. Filled squares indicate clonidine-treated group. Statistical analysis was done by Student's t-test. Ordinate : ambulatory activity and water drinking. Abscissa : time in hour.

Fig. 5 Effect of clonidine on brain stem norepinephrine turnover in SHR. Studies on norepinephrine turnover were carried out on third day of clonidine-treatment period. Diethyl dithiocarbamate (DDC) was given 2 and 4 hours before sacrifice. As shown in the lower part of this figure, rats were then sacrificed 3 and 12 hours after the 8:00 a.m. administration of clonidine (in the dark phase) on third day of the clonidine treatment period. Open column, age-matched control group; solid column, clonidine administered group. Ordinate: brain stem norepinephrine content.

produced a marked decrease in efferent nerve discharges of sympathetic adrenal nerve. Pre-treatment with phentolamine (100 μ g intracerebroventricular administration) prevented the decrease in sympathetic adrenal nerve activity induced by intracerebroventricular administration of clonidine in SHR.

Discussion

The most interesting observation in the present

experiment is that clonidine significantly depressed the ambulatory activity and water drinking of SHR at the beginning of the dark phase after the second drug administration. The decreased ambulatory activity induced by the second dose of clonidine in SHR may be a similar phenomenon to clonidine-induced sedation in patients with essential hypertension. There are several reports concerning the clonidine induced sedative effect in experimental animals¹³. Some

Fig. 6 Effect of clonidine on urinary catecholamine content in SHR. Before drug administration and two days thereafter, urinary samples for catecholamine assay were collected every 3 hours during the dark phase and once during the light phase. The urinary catecholamine content was expressed in terms of ng/mg of creatinine. The effect was tested in the two groups indicated by the following symbols: solid line, age-matched control group; dashed line, clonidine administered group. Each value shows mean $\pm$ S.E.. Statistical analysis was done by Student's t-test. Ordinate: urinary catecholamine content.

Fig. 7 Effect of clonidine on urine volume in SHR. Before drug administration and two days thereafter, urinary samples were collected every 3 hours during the dark phase and once during the light phase. Urine volume of each sample was measured. Each value shows mean $\pm$ S.E. Statistical analysis was done by Student's t-test.

investigators suggest that the clonidine-induced activation of α -adrenoceptors in the central nervous system produces sedation and hypotension⁵⁾⁽¹⁴⁾. The present investigation was designed around the proposed possibility that the decreased ambulatory activity induced by clonidine may be related to central and peripheral sympathetic nervous activity.

In this experiment, the lowering of brain stem norepinephrine produced by DDC appeared to be inhibited in clonidine-treated SHR at the beginning and the end of the dark phase. Clonidine decreased urinary norepinephrine excretion during the dark phase, but did not change it during the light phase. According to gross observation,

Fig. 8 Effects of clonidine on splanchnic nerve activity and sympathetic adrenal nerve activity in SHR. Each value shows mean $\pm$ S.E.. Statistical significance was tested by Student's t-test. Ordinate : spontaneous discharge rate of splanchnic nerve and sympathetic adrenal nerve. Each value is expressed in terms of % of control. Abscissa : time in minutes.

changes in urinary norepinephrine excretion were associated with the changes in ambulatory activity which were decreased by clonidine in the dark phase but not in the light phase. However, the time course changes in the decrease of urinary norepinephrine excretion induced by clonidine did not coincide with changes in ambulatory activity. Namely in spite of the increase of ambulatory activity at the end of the dark phase, urinary norepinephrine content decreased signifi-

cantly. Also, norepinephrine turnover in brain stem at the end of the dark phase was depressed the same as in the beginning of the dark phase. Hence, it is presumed that apparent increase of ambulation may be due to the increased drinking behavior at the end of the dark phase.

Previous studies in our laboratory revealed that clonidine (30 µg/kg, i.v.) induced a significant decrease both in efferent discharge rate of sympathetic adrenal nerve and in catecholamine

secretion from the adrenal medulla in adrenal venous blood¹⁵). These results suggested that clonidine depressed catecholamine secretion from the adrenal medulla by a centrally mediated mechanism in rats¹⁵. In this experiment, intravenous injection of clonidine also decreased the efferent nerve discharges of splanchnic nerve and sympathetic adrenal nerve in SHR. As sympathetic adrenal nerve and splanchnic nerve are both preganglionic sympathetic nerves, this finding indicates that clonidine depresses central sympathetic nerve activity.

Pretreatment with intracerebroventricular administration of phentolamine prevented the lowering phenomenon of efferent nerve discharges of sympathetic adrenal nerve brought about by intracerebroventricular administration of clonidine¹⁶). Therefore, if the clonidine induced sedation is associated with the activation of central α -adrenergic receptor, inhibitory effect of central sympathetic nerve activity induced by clonidine appears to be responsible for the clonidine-induced decrease in ambulatory activity in SHR.

On the other hand, the increased drinking behavior was observed at the end of the dark phase. Moreover, clonidine produced an increase in water drinking in the light phase which was not observed in the control period. With regard to the mechanism of dry mouth induced by clonidine in man, two factors have been principally considered: One is a possible inhibition of salivation¹⁷; the other is the inhibition of ADH secretion¹⁸). In the present experiment, it was observed that during the 3 hour period after clonidine administration at the beginning of dark phase, urine volume increased about five-fold, and thereafter it decreased significantly as compared with the control group. Therefore, the decreased humoral volume induced by diuresis appears to be re-

sponsible for the increased water intake after clonidine administration. In the present experiment, we did not study the direct action induced by clonidine on salivary flow. However, the decrease in the salivary flow has been observed in patients one hour after clonidine administration¹⁹). Accordingly, it is possible that the dry mouth may result from the decrease in salivary flow induced by clonidine in SHR. It was also presumed that drinking behavior which increased by clonidine may be masked by its strong sedative effect just after the second dose of clonidine administration at the beginning of the dark phase. These are problems to be solved in future investigations involving the assay for plasma ADH level in rats. Thus, the present findings suggest that decreased sympathetic nerve activity via a centrally mediated mechanism is at least in part associated with the decreased ambulatory activity induced by clonidine in SHR.

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