Spontaneously Hypertensive Rats
A Potential Model to Identify Drugs for Treatment of Learning Disorders

Alfredo Meneses, Enrique Hong

Abstract—Spontaneously hypertensive rats (SHR) of 3 to 12 months of age learned and retrieved less information than normotensive Wistar-Kyoto rats (WKY), although no difference was found with animals from 18 and 24 months of age. The combined influence of hypertension and aging had an additive detrimental effect on cognitive functions. Notwithstanding these deficiencies in learning and memory, SHR have seldom been used as a model in the screening of drugs with therapeutic potential for treatment of disorders of cognitive processes. Moreover, the calcium channel blocker nimodipine has beneficial effects on learning in both aged and hypertensive animals and humans. However, no attempt has been made to investigate whether nimodipine can reverse the additive deleterious effects of aging and hypertension in the same subject. We recently reported that deteriorated animals (middle-aged and/or hypertensive) chronically treated with nimodipine (via osmotic minipumps) exhibit higher learning scores. This information indicates that nimodipine can reverse the impairing effects of either aging or hypertension on learning; the presence of the two conditions, however, produces a severe impairment that can be partially reversed by this drug. Therefore, we propose that mature and middle-aged SHR represent a model for the screening of potentially useful drugs in the treatment of learning disorders, probably associated with hypertension and/or aging. Nevertheless, it must be remembered that the SHR is a genetic model and the appearance of neural disturbances could be a parallel genetic phenomenon and not necessarily or exclusively related to hypertension per se. (Hypertension. 1998;31:968-972.)

Key Words: rats, inbred SHR ■ learning ■ memory ■ aging ■ pharmacology ■ models

A great expansion of experimental research began with the development of rat strains with genetically inherited hypertension. The SHR offers specific and uniform genetic predisposition,1 thus allowing the study of the causes, mechanisms, and pathology of hypertension, as well as its behavioral consequences, and the comparison of the efficacy of proposed therapeutic interventions in relation to existing clinical treatments. Moreover, central neurohormonal mechanisms constitute the dominating trigger influence in SHR1 and provide a model of hypertension that allows the study of the combined influence of both aging and hypertension on cognitive and physical functions on different developmental stages.2,3 Notwithstanding, the SHR is a genetic model, and the appearance of neural disturbances could be a parallel genetic phenomenon and not necessarily or exclusively related to the elevated blood pressure per se. Normal aging also produces a slow decline in neuron population, tissue distensibility, basal metabolic rate, and oxygen consumption, thus affecting cardiovascular performance. The aim of the present work was to present evidence obtained in our laboratory supporting the idea that the SHR is a suitable behavioral model to test drugs with potential therapeutic usefulness for the treatment of behavioral and neural disturbances induced by hypertension and aging.

Methods
As previously reported,2,4,5 modular operant chambers (Coulbourn Instruments) were used; each chamber was enclosed in a sound-attenuating compartment equipped with a ventilating fan. A retractable lever was mounted 4 cm above the floor and 10 cm from right and left walls. A food magazine for pellets (Bio Serv) was located 5 cm to the right of the lever and 3 cm above the floor. A house light was located in the right top corner 29 cm from the floor. Solid-state programming equipment was used for control and recording (Coulbourn Instruments).

Training
Each rat was placed into an experimental chamber and allowed to acclimatize to the experimental environment. The animals were left in the chambers until they were able to find and eat 45 food pellets (45 mg each pellet); all pellets were available simultaneously. Immediately afterward, the trial began and there was an intertrial interval of 60 seconds. The former consisted of the presentation of an illuminated retractable lever for 8 seconds (conditioned stimulus, CS) followed immediately by delivery of a food pellet (unconditioned stimulus, US). Each time the animal pressed the retractable lever (CS) was considered as a conditioned response (CR); when this occurred the trial was shortened and the lever retracted, the light was turned off, and food (US) was also immediately delivered. An increase or decrease in percentage of CR was considered as an enhancement or impairment in the consolidation of learning. The first session consisted of 10 trials; later sessions consisted of 20 trials. One month later, animals were retrained under the same conditions. In this case the increase in the percentage of CRs was
considered an event related with reacquisition or memory.2,3 All animal facilities and protocols were approved by the local committee for animal research (CINVESTAV-IPN).

Body Weight and SBP
Body weight and SBP measurements were made 1 week before the autoshaping task sessions. SBP was measured by tail-cuff plethysmography in animals already habituated to this manipulation.

Statistical Analysis
The CRs were transformed to percentage of total trials for each session. CR%, SBP, and heart rate were analyzed using ANOVA for repeated measures (eg, strain x treatment) followed by additional post hoc comparisons using Tukey’s t test. In all statistical comparisons, P < .05 was used as the criterion for significance.

Animals
Groups of male SHR and their respective control WKY of several ages (3, 6, 9, 12, 18, and 24 months old) were used. Groups of each age and strain were housed separately in a temperature- and light-controlled room under a 12-hour light/dark cycle, with water and food provided ad libitum. Because the autoshaping task used food administration for conditioning, 1 week before autoshaping training and retraining, the animals were gradually deprived of food for 7 days so that their body weights were reduced by 15%: this limited body weight reduction allowed the exclusion of any disproportionate effect induced by food deprivation. The experiments consisted of two phases: (1) autoshaping training (acquisition) lasting for six daily sessions, with each training session separated by 24 hours; and (2) autoshaping retraining (reacquisition) consisting of a series of sessions identical to that of autoshaping training (performed 1 month later). The number of animals per group was 6 to 12; they were naive to the autoshaping testing procedure and used only once. The smaller groups of animals correspond to the older rats, since they were difficult to obtain.

Drug Treatment
Four groups of animals were randomly assigned to each treatment. Rats were anesthetized with sodium pentobarbitone (30 to 40 mg/kg IP) 30 minutes before surgery. The dorsal area was scrubbed with iodine solution, shaved, and wiped with 70% ethanol solution. A ventral midline incision, approximately 1.5 cm in length, was made through the skin, and a sterilized Alzet osmotic minipump (model 2ML4; mean pumping rate of 2.38 ± 1 μL/h during 28 days) was inserted through the incision. A suture was made in the skin, and the animals were returned to their home cages for complete recovery from anesthesia; at least 16 hours were allowed to elapse for this purpose. As previously reported,1 WKY and SHR of 12 months of age were implanted with subcutaneous osmotic minipumps releasing nimodipine (0.4 mg/kg per day). After 3 weeks of treatment, vehicle- and nimodipine-treated animals received daily autoshaping training sessions during 4 days. The continuous infusion with osmotic minipumps was selected because these pumps provide a sustained and stable administration of low light-protected doses of the drug. Moreover, this administration protocol allows the study of a possible direct effect of nimodipine on learning unrelated to its hypotensive effects, thus avoiding drastic and long-lasting changes in blood pressure and resembling a slow-release oral dose for humans. The

| TABLE 1. Acquisition and Reacquisition of Percentage of CR* Obtained Through Autoshaping Learning Task From SHR and WKY Rats of Different Ages |
|-----------------|-----------------|-----------------|-----------------|
| Age, mo         | WKY             | SHR             | WKY             |
| 3               | 97 ± 2          | 68 ± 4†         | 48 ± 7          |
| 6               | 96 ± 1          | 49 ± 3†         | 58 ± 5          |
| 9               | 82 ± 5          | 34 ± 9†         | 52 ± 8          |
| 12              | 65 ± 8          | 19 ± 9†         | 9 ± 4           |
| 18              | 16 ± 3          | 10 ± 3          | 19 ± 3          |
| 24              | 8 ± 4           | 5 ± 3           | 11 ± 3          |

*Mean ± SEM, maximal score obtained after six training sessions. †P < .05, by Tukey’s t test comparing SHR vs WKY of the same age. Data reproduced with permission.2,4

number of animals in these groups was 7; they were naive to the autoshaping testing procedure and used only once.

Results
Effects of Hypertension and Age on Cognitive Processes
The autoshaping learning task has proved to be valuable in the study of the joint action of stimulus-stimulus (ie, classic conditioning) and response-stimulus (ie, instrumental-operant conditioning).6–8 Using the autoshaping task, an age-related decrease in learning and memory was found, ie, there were significant differences between strains (F[1,1236] = 64.49, P < .0001), ages (F[5,1236] = 61.23, P < .0001), and sessions (F[11,111] = 33.07, P < .0001), except in the case of the 18- and 24-month groups. The reductions in learning and memory were greater in the younger groups (3 to 12 months old) of SHR (Table 1). This suggests that the detrimental effects of hypertension may be additive with age. The youngest (3 months old) WKY rats showed the greatest ability to learn; in contrast, the lower rate of learning was shown by 24-month-old animals. With respect to the reacquisition ability2 (an index of memory), a similar age-related influence was observed. Hence, the level of conditioning in the 3-month-old WKY rats was significantly higher than that obtained by the 12-month-old group of the same strain. This finding suggests a better retention in the younger group, whereas SHR displayed smaller conditioning scores during retraining. In the case of SHR, the scores after training sessions were lower than those observed with WKY; however, the number of conditioned responses obtained after 1 month without any session suggests a higher practice effect in SHR than in WKY (except in the case of the youngest SHR of 3 months old).

Regarding the effects of hypertension, data from the 3-, 6-, and 9-month-old groups provided further support to the suggestion that this pathological condition impairs learning and memory6,9–12 and may accentuate age-related brain damage on these cognitive functions.7 If one considers the differences between WKY and SHR, they fluctuate but tend to increase with age. For instance, if WKY values are divided by SHR values, there is a steady increase in the differences between WKY and SHR: 1.4-fold at 3 months and increasing to 3.4-fold at 12 months (Table 1).
TABLE 2. Effect of Nimodipine (0.4 mg/kg per d) Obtained During the Last Autoshaping Session in Normotensive WKY and SHR Rats

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>Vehicle CR, %</th>
<th>SHR CR, %</th>
<th>Nimodipine Vehicle CR, %</th>
<th>SHR CR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>20±5</td>
<td>7±1†</td>
<td>37±6†</td>
<td>26±2‡</td>
</tr>
</tbody>
</table>

*Mean±SEM, maximal score obtained after four training sessions from 7 animals per group. P<.05 by Tukey's t-test; † vs WKY control vehicle, ‡ vs SHR control genetic.

Effect of Nimodipine on SBP and Heart Rate

The analysis of heart rate and SBP revealed significant differences between both strains (F[3,139]=596.36, P<.001, and F[3,139]=805, P<.005, respectively), as well as between treatments (F[2,139]=25.07, P<.05 and F[4,139]=46.02, P<.05). For instance, both heart rate and SBP were significantly higher in SHR with respect to WKY (443±1 and 360±1 bpm and 207±3 and 141±1 mm Hg, respectively). The treatment with nimodipine produced a significant decrease in SBP accompanied by a significant increase in heart rate in both strains during the first week; however, these changes persisted only in WKY, since in SHR there was a gradual increase in SBP that almost reached the control level during the second week.

Learning

The results showed that there were significant differences between strains (F[3,111]=5.63, P<.001), treatments (F[3,111]=2.71, P<.05), and sessions (F[3,111]=2.16, P<.05). The maximal differences were observed during the last session (Table 2), showing that the rate of CR increased significantly in each treated group (F[3,15]=8.30, P<.001). Both animals, WKY and SHR, dosed with nimodipine displayed higher rates of CR than those receiving only the vehicle. Furthermore, nimodipine-treated WKY reached the highest score while the control SHR group displayed the lowest score. The increment in CR in nimodipine-treated SHR was 3.7-fold in relation to the control group, whereas in WKY, the nimodipine-treated animals showed a 1.85-fold increase in CR (Table 2). These results are in accordance with previous data showing the ability of nimodipine to improve learning in either aged or hypertensive animals. Notwithstanding, the present data from middle-aged normotensive WKY and SHR may further suggest that the presence of aging and hypertension produces additive cognitive alterations that can be partially reversed by nimodipine.

Discussion

Cognitive Deficits Induced by Hypertension and Aging

Repeated exposure to a novel environment leads to a reduction in activity; eg, during the first recording session of spontaneous activity, no significant differences between WKY and SHR are usually noticed. During the second session, however, SHR are more active than WKY and the age-related decrement in activity is not further observed in WKY and SHR older than 12 months, suggesting a higher rate of habituation to a novel environment in mature animals and a decreased activity associated with senescence.

Hypertensive elderly people have shown impairment in learning and memory that has been attributed to cognitive dysfunctions and neural alterations, probably related to hypertrophy of cerebral vessels and reduced cerebral blood flow. In the case of SHR, diverse authors have reported hyperactivity, hyperreactivity, lower levels of anxiety, and a decrement of habituation capabilities as well as severe deficit in attention and decreased learning ability in different behavioral tasks. Therefore, the use of the autoshaping test as a nonthreatening behavioral task is completely justified.

Nimodipine Reverses Cognitive Deficits

The idea that hypertension may cause further dysfunctional changes complicating those induced by aging is supported by two observations in the autoshaping learning task: (1) normotensive WKY from 3 to 12 months of age always showed significantly higher scores of learning than the corresponding SHR groups (Table 1), and (2) the nimodipine-treated WKY group consistently displayed the highest rates of learning (Table 2). Based on the latter observation, one could suggest that the score of learning exhibited by the nimodipine-treated WKY group is very similar to those displayed by animals not affected by aging or hypertension. It is worth highlighting that the performance of the SHR group treated with nimodipine reached a substantially greater effect in SHR than WKY (3.7-fold versus 1.85-fold, respectively); this suggests that nimodipine was able to partially reverse the impairment associated with aging and hypertension. The nimodipine-induced increase in CR does not seem to be due to the decrease in SBP, since the enhancing effects of nimodipine in SHR, whose SBP had returned to baseline values before performance of the autoshaping task, may point toward a direct effect of this drug on the central nervous system. This possibility is supported by previous studies showing that nimodipine, given at doses and routes of administration causing no hypotensive effects, has clear beneficial effects on learning. Although several routes have been tested for repeated administration of nimodipine, the continuous infusion with osmotic minipumps was selected because it provides a sustained and stable administration of low (light-protected) doses of the drug. Indeed, our administration protocol was designed to test a possible direct effect of nimodipine on learning unrelated to its hypotensive effects, thus avoiding drastic and long-lasting changes in blood pressure and to approximating a slow-release oral dose for humans. Because nimodipine was not altered by exposure to the body temperature during 4 weeks, the behavioral and cardiovascular changes observed in the present study are due to drug treatment. With use of the multiple variable interval extinction test in 3-month-old SHR, but not in WKY of the same age, an improving effect of
nimodipine on learning was detected, this lack of effect in WKY could have been due to the age of the animals (3 months). In fact, we found that 3-month-old but not middle-aged (12-month-old) WKY display full ability to learn.3,7 Thus, despite evident experimental differences such as the age of animals (12 months versus 3 months), administration design (infusion versus intubation), dose (0.4 mg/kg per d versus 9 mg/kg total), and behavioral task (autoshaping versus multiple variable interval extinction), it seems that the presence of either aging or hypertension or both is a requisite for the manifestation of the beneficial effects of nimodipine on learning. Because vehicle- and nimodipine-treated SHR showed the highest difference in the values of CR while the corresponding WKY groups displayed only a moderate (although significant) difference, nimodipine-treated WKY reached the highest score, whereas the control SHR group displayed the lowest one. The SHR represents a genetic model, and it is unknown whether neural and behavioral disturbances could be a parallel genetic phenomenon and not causally related to the elevated blood pressure per se. Nevertheless, it is interesting that deficits in cognitive processes have been also observed in stroke-prone SHR and SHR developed by Koletsky (Golda and Petr). Because hypertension does not develop in the SHR until about 2 months of age, it would be interesting to compare the performance between young normotensive SHR and WKY. Moreover, it is unclear whether any drug that lowers blood pressure in the SHR to normotensive values could improve learning and memory. Nevertheless, it was recently reported that blood pressure reduction by propranolol but not captopril has an adverse effect on cognitive function (assessed with the Morris water maze) in hypertensive rats.

A perturbation of the normal Ca2+ metabolism leading to its increased intraneuronal concentration appears to be an important factor correlated with both age-related physiological deficits and learning and memory deficits. In this context, it is accepted that hypertrophy of cerebral vessels and a consequent reduction in blood flow to certain critical areas in the brain may be involved, although this mechanism may also account for the manifestation of the beneficial effects of nimodipine on learning. Because vehicle- and nimodipine-treated SHR showed the highest difference in the values of CR while the corresponding WKY groups displayed only a moderate (although significant) difference, nimodipine-treated WKY reached the highest score, whereas the control SHR group displayed the lowest one. The SHR represents a genetic model, and it is unknown whether neural and behavioral disturbances could be a parallel genetic phenomenon and not causally related to the elevated blood pressure per se. Nevertheless, it is interesting that deficits in cognitive processes have been also observed in stroke-prone SHR and SHR developed by Koletsky (Golda and Petr). Because hypertension does not develop in the SHR until about 2 months of age, it would be interesting to compare the performance between young normotensive SHR and WKY. Moreover, it is unclear whether any drug that lowers blood pressure in the SHR to normotensive values could improve learning and memory. Nevertheless, it was recently reported that blood pressure reduction by propranolol but not captopril has an adverse effect on cognitive function (assessed with the Morris water maze) in hypertensive rats.

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