Synaptic plasticity in sympathetic ganglia from acquired and inherited forms of ouabain-dependent hypertension

AZEEZ A. AILERU,* ALINE DE ALBUQUERQUE,* JOHN M. HAMLYN, PAOLO MANUNTA, JUI R. SHAH, MATTHEW J. HAMILTON, AND DANIEL WEINREICH

Departments of Pharmacology and Physiology, University of Maryland, School of Medicine, Baltimore, Maryland 21201-1559

Received 6 June 2000; accepted in final form 16 April 2001

Aileru, Azeez A., Aline De Albuquerque, John M. Hamlyn, Paolo Manunta, Jui R. Shah, Matthew J. Hamilton, and Daniel Weinreich. Synaptic plasticity in sympathetic ganglia from acquired and inherited forms of ouabain-dependent hypertension. Am J Physiol Regulatory Integrative Comp Physiol 281: R635–R644, 2001.—Altered sympathetic nervous system activity has been implicated often in hypertension. We examined short-term potentiation [posttetanic potentiation (PTP)] and long-term potentiation (LTP) in the isolated superior cervical ganglia (SCG) from Sprague-Dawley (SD) rats given vehicle, digoxin, or ouabain by subcutaneous implants as well as in animals with ouabain-induced hypertension (OHR), and inbred Baltimore ouabain-resistant (BOR) and Baltimore ouabain-sensitive (BOS) strains of rats. Postganglionic compound action potentials (CAP) were used to determine PTP and LTP following a tetanic stimulus (20 Hz, 20 s). Baseline CAP magnitude was greater in ganglia from OHR than in vehicle-treated SD rats before tetanus, but the decay time constant of PTP was significantly decreased in OHR and in rats infused with digoxin that were not normotensive. In hypertensive BOS and OHR, the time constants for the decay of both PTP and LTP (tL) were increased and correlated with blood pressure (slope = 0.15 min/mmHg, r = 0.52, P < 0.047 and 6.7 min/mmHg, r = 0.906, P < 0.0001, respectively). In BOS and OHR, tL (minutes) was 492 ± 40 (n = 7) and 539 ± 41 (n = 5), respectively, and differed (P < 0.05) from BOR (257 ± 48, n = 4), SD vehicle rats (240 ± 18, n = 4), and captopril-treated OHR (370 ± 52, n = 5). After the tetanus, the CAP at 90 min in BOS and OHR SCG declined less rapidly vs. SD vehicle rats or BOR. Captopril normalized blood pressure and tL in OHR. We conclude that the duration of ganglionic LTP and blood pressure are tightly linked in ouabain-dependent hypertension. Our results favor the possibility that enhanced duration of LTP in sympathetic neurons contributes to the increase in sympathetic nerve activity in ouabain-dependent hypertension and suggest that a captopril-sensitive step mediates the link of ouabain with LTP.

neurons; angiotensin; breeding; sodium pump; captopril; long-term potentiation

INCREASED SYMPATHETIC NERVE ACTIVITY (SNA) has been implicated in the development and maintenance of several forms of human and experimental hypertension (2, 8, 13, 18, 24, 26, 34, 35, 38, 43). Previous studies have shown alterations in ganglionic function in hypertensive animals (26, 46). For example, postsynaptically, spike frequency adaptation is curtailed in the spontaneously hypertensive rats (SHR) model (46). Presynaptically, there is enhanced release of acetylcholine in SHR (27). Together, these changes in ganglionic function may contribute to the increased SNA. Of particular relevance to sustained activity of the sympathetic nerves is the phenomenon known as long-term synaptic potentiation (LTP). This form of activity-dependent synaptic plasticity is a presynaptic phenomenon in mammalian sympathetic ganglia. It appears to require an elevated calcium concentration in the presynaptic nerve terminal, and its induction and maintenance require activation of 5-hydroxytryptamine (5-HT3) receptors (1, 5).

Recently, ouabain or a closely related isomer has been found in the circulation and tissues of humans, rats, and cattle (14, 15, 23, 42). The circulating level of this “endogenous ouabain” (EO) appears to depend on adrenocortical function (4, 25) and is elevated in a large portion of patients with essential hypertension (32, 41). Moreover, the prolonged administration of ouabain in normal rats increases the circulating and hypothalamic levels of ouabain (31). The highly polar ouabain molecule probably enters the hypothalamic region via the fenestrated epithelia adjacent to the circumventricular organs (20) and, by enhancing SNA, evokes sustained elevations of blood pressure (16–18, 30, 47). One possibility is that the mechanism of the heightened sympathoexcitation in ouabain-dependent hypertension could reflect altered membrane electrical activity and/or transmitter release from preganglionic neurons (8, 21). Increased preganglionic activity would be expected to recruit larger numbers of postganglionic autonomic neurons to excitation (10, 26). In addition, the recruitment mechanism itself can be enhanced by alterations in membrane properties that may augment the firing of postganglionic neurons (21, 46).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpregu.org 0363-6119/01 $5.00 Copyright © 2001 the American Physiological Society
In the present investigation, we examined the synaptic efficacy of autonomic ganglionic transmission in the superior cervical ganglia (SCG) in an acquired model of ouabain-dependent hypertension. In addition, we took advantage of two new strains of rats that were developed in Baltimore from a colony of outbred Sprague-Dawley (SD) rats. The strains were isolated initially based on their heightened susceptibility [Baltimore ouabain-sensitive (BOS)] or resistance [Baltimore ouabain-resistant (BOR)] to the hypertensinogenic effect of 5-wk infusions of ouabain. With continued selection and inbreeding after the F3 generation, the heightened sensitivity of the BOS strain led to the appearance of spontaneous hypertension appearing before 7 wk of age. At that stage, the blood pressure in BOS rats was not augmented by infusion of exogenous ouabain. In contrast, the counter strain of rats (BOR) was invariably normotensive, and there was no increase in blood pressure during the ouabain infusion.

The rat models we describe in the present work may be of particular relevance for exploring the mechanisms of human essential hypertension. For example, the prolonged administration of ouabain in otherwise normal and healthy outbred rats induces sustained hypertension, and the animals exhibit plasma levels of ouabain that mimic those observed in patients with essential hypertension (31, 32, 41). Second, rats with ouabain-dependent hypertension have elevated total peripheral vascular resistance, “normal” circulating renin activity, increased brain angiotensinogen, and elevated renal nerve traffic (17–19, 31, 47, 49). From these and other studies, it has been suggested that this model of high blood pressure reproduces many of the key hemodynamic and endocrine features of the patients described above.

The availability of inherited and acquired forms of ouabain-dependent hypertension offers many unique advantages compared with other contemporary rat models. Specifically, the presence and absence of elevated circulating levels of the primary initiating agent, i.e., ouabain in this instance, allow for the dissection of primary vs. secondary effects of this steroid on a variety of physiological parameters. When taken together with parallel studies with digoxin, the models used enable the significance of the relationships among the circulating agents, mechanisms of ganglionic function, and long-term blood pressure levels to be determined more readily.

MATERIALS AND METHODS

Outbred rats. All animal use-related protocols were reviewed and approved by the University of Maryland Institutional Animal Care and Use Committee.

Normotensive male outbred SD rats (Harlan, Indianapolis, IN) weighing 200–250 g received either a 21-day slow-release pellet (Innovative Research, Sarasota, FL) containing ouabain (0.25 mg), digoxin (0.5 mg), or no addition (vehicle) subcutaneously.

While the rats were under halothane anesthesia, the flank was shaved and cleaned with iodine solution. A skin incision was made, and the pellet was inserted subcutaneously. Wound clips were used to close the incision. The entire surgical procedure took ~5 min, and all animals recovered rapidly following the procedure.

In some studies, captopril (3 mg/day) was given in the drinking water. Tail-cuff blood pressures were measured (weekly) before and on days 14 and 21 following implantation. Direct recordings of mean arterial pressure (MAP) were obtained from some animals to verify hypertension.

Inbred rats. Two new strains of rats were isolated in Baltimore based on their characteristic resistance or sensitivity to the hypertensinogenic effect of ouabain. The strains were developed simultaneously from an F0 generation comprising 80 normotensive outbred SD rats (40 males, 40 females) obtained from Harlan. Each member of the F0 generation received a maximal pressor dose of 30 μg·kg⁻¹·day⁻¹ of ouabain octahydrate (Sigma Chemical, St. Louis, MO) infused subcutaneously via a miniosmotic pump (ALZET 2002, ALZA) for 5 wk as previously described (30). Ouabain was dissolved in sterile phosphate-buffered saline to yield an infusion dose of 30 μg·kg⁻¹·day⁻¹, assuming a mean pump rate of 12 μl/day. The miniosmotic pumps were equilibrated in sterile phosphate-buffered saline overnight before implantation (30).

Systolic and mean arterial blood pressures were measured at weekly intervals in conscious rats by tail-cuff plethysmography. In response to the 5-wk infusion of ouabain, ~92% of the F0 rats developed sustained hypertension and were designated ouabain sensitive (BOS). The remaining rats showed minimal or no increase in systolic and mean blood pressures and were designated as ouabain resistant (BOR). The F1 generations of BOS and BOR were derived by sib mating of F0 rats with the highest sensitivity or resistance to ouabain, respectively. A similar experimental, selection, and mating protocol was repeated through the F2–F8 generations. In this study, animals from the F5–F8 generations were used; there being no significant differences in the measured parameters among these generations. Most of the data were obtained from the F6 and F7 generations. The inbred rats were 12 wk old at the time of death.

Blood pressure measurements. Indirect systolic and mean arterial blood pressures were recorded by tail-cuff plethysmography using a commercial photoelectric system (model 29 blood pressure meter/amplifier; IITC, Woodland Hills, CA) and a device providing constant rates of cuff inflation and deflation. The output from both devices was recorded on a polygraph. In this procedure, conscious rats were restrained in acrylic animal holders for 5–10 min in a warm, quiet room, and they were conditioned to numerous cuff inflation-deflation cycles. The onset of oscillations and the maximum amplitude observed during cuff deflation with this system were taken as the systolic and mean arterial blood pressures, respectively (9). At the end of each experiment, direct MAPs were measured via femoral catheters as previously described (3).

Preparation of plasma and tissue. Animals were killed by decapitation. Trunk blood was collected into heparinized containers containing EDTA. The blood was centrifuged at 3,000 g, and the clear plasma was removed and stored at −20°C until analysis.

Both SCG along with their pre- and postganglionic nerve trunks were dissected. The ganglia were submerged immediately in ice-cold (5°C) Locke solution whose composition was (in mM): 136 NaCl, 5.6 KCl, 1.2 NaHCO₃, 1.2 NaH₂PO₄, 2.2 CaCl₂, 1.2 MgCl₂, 10 dextrose, and 0.03 cho-
line chloride, equilibrated continuously with 95% O2-5% CO2, pH 7.2–7.4. All ganglia were used within 2 h of dissection.

Each SCG was desheathed, trimmed of adhering connective tissue and blood vessels, and pinned to the Sylgard (Dow Corning, Midland, MI)-coated floor of a recording chamber (~0.25-ml volume). Ganglia were superfused (1–2 ml/min) with oxygenated Locke solution at 22–24°C delivered via a peristaltic pump or by gravity, and the Locke solution exited via a hole in the floor of the recording chamber. A thermostor (Thermometric, Edison, NJ) positioned ~2 mm from the SCG monitored bath temperature. Unless otherwise noted, hexamethonium (100–300 μM) was added to the Locke solution and superfused over ganglia for at least 60 min before application of tetanic stimuli.

Electrophysiological recordings. Extracellular recordings of the postganglionic compound action potential (CAP) were measured with a bipolar suction electrode placed on the internal carotid nerve. The suction electrode was connected to the input stage of an alternating current-coupled differential preamplifier (0.1–1.0 kHz; World Precision Instruments; model 2300). Data were filtered at 1 kHz and, when necessary, sampled at 10 kHz. The cerebral sympathetic trunk (CST) was threaded through a Vaseline-lined notch into a mineral oil-containing compartment and placed onto two platinum-stimulating electrodes. CAPs were evoked by supramaximal (twice the current strength necessary to elicit a maximal postganglionic CAP) electrical stimulation applied to the CST. Most recordings were made under conditions where the operator was unaware of the phenotype or genotype of the animals.

Measurement of spike frequency accommodation was performed with conventional “sharp” micropipettes as described by Yarowsky and Weinreich (46). Current-clamp recordings were made with an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) either in bridge (filtering at 10 kHz) or in discontinuous mode (sample rate 5 kHz, filtering at 3 kHz).

Assay for ouabain, EO, and digoxin. Measurements of plasma ouabain and EO were made on C-18-extracted plasma using a polyclonal ouabain antiserum (R68) validated for the detection of ouabain and EO (14). The antiserum was used in the radioimmunoassay format as described (41). The assay shows no significant cross-reactivity with the common adrenocortical, ovarian, or testicular steroids. The ouabain antiserum shows ~5% cross-reactivity to digoxin so that differential elution from the C-18 extraction columns was employed to separate ouabain and EO from digoxin during sample preparation. Plasma digoxin was measured using a commercial kit (Diagnostic Products, Los Angeles, CA). The digoxin assay showed <2% cross-reactivity with unextracted ouabain.

Data analysis. The effect a brief preganglionic supramaximal tetanic stimulus (20 Hz, 20 s) on synaptic transmission was assessed by measuring changes in the peak-to-peak amplitude of the evoked CAP. Alterations in the posttetanic peak amplitude of the CAP were taken as an index of the number of postganglionic neurons synaptically excited to spike threshold. With the use of pClamp software (Axon Instruments), values of peak-to-peak amplitude were determined by positioning mouse-controlled cursors on digitized records of the CAP; one cursor was placed after the stimulus artifact just before the initial rising phase. The other was usually located at a point where the spike had returned to within 20% of the baseline. The baseline CAPs were evoked at 0.2 Hz, and 12 CAPs were collected and averaged via pClamp software (Axon Instruments) running on an IBM PC XT with a TL1 interface. The 0.2-Hz presynaptic rate of stimulation was chosen to minimize synaptic depression (36) and activity-dependent synaptic potentiation (6, 7, 12, 48). The postganglionic response was made approximately one-half the maximal peak amplitude by the addition of a nicotinic antagonist hexamethonium (100–300 μM; see Ref. 7). After the CAP amplitude had declined to a steady-state pretetanic baseline in the presence of hexamethonium (usually 45–60 min), CAPs were monitored for another 10 min before tetanic stimulation. The posttetanic change in CAP was calculated as a percent change of maximum potentiation relative to the control prestimulus CAP amplitude. Once a steady state was reached, the effects of hexamethonium on the steady-state pretetanic baseline did not vary with time, and there was no further change in the CAP in the presence of hexamethonium.

The IBM PC XT was connected to a Dell System 320 (20 MHz, 386 PC) via a two-node network for monitoring the peak-to-peak amplitude and integral CAP responses “online.”

Statistical analyses. Data were expressed as means ± SE. Differences between multiple means were analyzed using analysis of variance, and sample means were compared using Fisher’s specific test. Significance was taken as P < 0.05. Simple linear regression was used to evaluate the relationships among the various parameters where appropriate.

Reagents. All salts and agents were purchased from Sigma Chemical.

RESULTS

Cardiovascular responses in outbred rats. Table 1 shows blood pressures, plasma levels of ouabain, and digoxin immunoreactivity in outbred SD rats that were given vehicle, ouabain, or digoxin for the indicated periods. The circulating levels of ouabain and MAP were elevated only in the rats that received continuous infusions of ouabain for several weeks (OHR). Short-term (3–5 days) continuous infusions of ouabain and/or the prolonged administration of digoxin had no effect on MAP even though the circulating levels of the respective steroids were elevated to a similar extent. Only minimal amounts of digoxin immunoreactivity were detected in the vehicle and ouabain-infused rats.

Characterization of ganglionic synaptic transmission in outbred rats and the effect of ouabain and digoxin.

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
<th>Plasma Ouabain Immunoreactivity, nmol/l</th>
<th>Plasma Digoxin Immunoreactivity, nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD + vehicle 3 wk (13)</td>
<td>108 ± 2.4</td>
<td>0.63 ± 0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SD + ouabain 3–5 days (8)</td>
<td>106 ± 5.1</td>
<td>5.51 ± 0.9†</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SD + ouabain 3 wk (7)</td>
<td>122 ± 1.2*</td>
<td>4.56 ± 0.8†</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SD + digoxin 3 wk (8)</td>
<td>105.5 ± 2.6</td>
<td>0.83 ± 0.3</td>
<td>3.97 ± 1.3*</td>
</tr>
</tbody>
</table>

Values are means ± SE with the number of animals studied in parentheses. Animals were studied at 6 mo of age. Mean arterial pressure (MAP) was obtained immediately before death. *P < 0.005 vs. other groups. †P < 0.001 vs. vehicle and digoxin-infused groups. SD, Sprague-Dawley rats.
We assessed the efficacy of synaptic transmission in isolated rat SCG by measuring changes in the amplitude of postganglionic CAP induced by a standard brief preganglionic tetanus (20 Hz, 20 s). This stimulus was chosen because it elicits both short-duration [posttetanic potentiation (PTP)] and long-term potentiation (LTP) of nicotinic synaptic transmission (6). In the rat SCG, PTP lasts for several minutes, whereas LTP is maintained for tens of minutes to many hours. The time constants of the decay of these components can be determined by the sum of two exponential terms with time constants, \( t_p \) and \( t_L \), providing a qualitative description of the persistence of PTP and LTP, respectively (7). We performed our experiments at 22–24°C. At temperatures >32°C, one-half of the preparations showed no detectable decay of LTP so that the kinetics of LTP were not quantifiable within the time course of the typical experiment (6).

Figure 1 shows representative recordings of postganglionic CAPs from SD vehicle rats and OHR before, immediately following tetanic stimulation, and 30 min after the tetanus. The amplitude of PTP was greater in the ganglia from the vehicle-infused rats than in OHR ganglia, but LTP was more sustained in the OHR ganglia. In the vehicle-infused rats, the mean amplitude of the baseline CAP was \( \mu \text{V} \); in OHR and in digoxin-infused rats, the mean amplitude of the baseline CAP was marginally elevated relative to SD vehicle rats, and the maximal amplitude of PTP was reduced to \( \mu \text{V} \) (\( P \), 0.01 vs. SD vehicle) and 3.6 \( \mu \text{V} \), respectively. There was no significant correlation between the maximal amplitude of PTP and the pretetanus baseline.

Figure 2 presents the group data normalized as percent change relative to the control, pretetanic, response. Some rats were infused with ouabain for 3–5 days (PRE-HYP) and were normotensive at death. Number of animals used is in parentheses. See Table 1 for other details. Error bars are SE. *\( P \) < 0.02; **\( P \) < 0.005 vs. control.

Fig. 1. Two forms of synaptic plasticity, posttetanic potentiation (PTP) and long-term potentiation (LTP), recorded in the superior cervical ganglion (SCG) in vitro in outbred rats. Top, A–C and D–F: representative postganglionic recordings of compound action potentials (CAPs) evoked by presynaptic stimulation at 0.2 Hz from the ganglia of normotensive Sprague-Dawley (SD) vehicle rats and ouabain-induced hypertensive SD rats (OHR), respectively. Each record is the average of 12 CAPs. Bottom: amplitude of ganglionic CAPs before tetanus (20 Hz, 20 s) and their decay with time in SD vehicle rats (left) and OHR (right). Letters show times at which the representative postganglionic recordings were taken.

Fig. 2. Effect of cardiac glycosides on potentiation of PTP in SCG and mean blood pressure (MAP) in outbred rats. SD rats were administered vehicle, ouabain, or digoxin for 3 wk. MAPs were measured, and the SCG were removed and prepared for recording. The maximum amplitude of the PTP results is presented as the percent change relative to the control, pretetanic, response. Some rats were infused with ouabain for 3–5 days (PRE-HYP) and were normotensive at death. Number of animals used is in parentheses. See Table 1 for other details. Error bars are SE. *\( P \) < 0.02; **\( P \) < 0.005 vs. control.
of antihypertensive therapy. Table 2 shows blood pressure and HR, ganglionic parameters, and circulating levels of ouabain immunoreactivity recorded in BOR, BOS, OHR, captopril-treated OHR, and vehicle-treated SD rats. The average MAP and HR in BOR were not significantly different from values recorded in normal age-matched outbred rats given vehicle or those in captopril-treated OHR. In BOS and OHR, MAP was significantly elevated vs. BOR or SD vehicle rats. BOS rats had higher HRs than BOR, SD vehicle rats, or captopril-treated OHR. HRs tended to be higher also in OHR vs. SD vehicle rats or BOR, but the difference did not achieve statistical significance ($P = 0.06$).

In BOS and BOR, the application of a brief preganglionic tetanus (20 Hz, 20 s) elicited a vigorous and sustained increase in the size of the evoked CAP in preparations from outbred rats. The traces illustrated in Fig. 3A show the prototypic CAPs recorded before, 1 and 90 min after the tetanus. In SCG from BOR, potentiation was maximum ~1 min after the tetanus and declined to near control values by 90 min. In contrast to the response of the BOR ganglia, preparations from BOS rats showed marked increases in the longevity of the potentiated CAP (Fig. 3A). Ninety minutes after the tetanus, the CAP recorded from BOS ganglia remained nearly twice the size of the pretetanic CAP. The maximum potentiation of the CAP relative to control appeared qualitatively similar in BOR and BOS.

The time course of the potentiated CAP, plotted as CAP amplitude as percent of maximum amplitude, is shown in Fig. 3B for all groups of animals at 30, 60, and 90 min following the tetanus. Values of 594, 538, 184, 282, and 190 min were derived for the time constant of decay of posttetanic potentiation; $t_{L}$, time constant of decay of long-term potentiation; BOS, Baltimore ouabain-sensitive rats; BOR, Baltimore ouabain-resistant rats; OHR, ouabain-induced hypertensive SD rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>$t_{P}$, min</th>
<th>$t_{L}$, min</th>
<th>Plasma Ouabain Immunoreactivity, nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOS (7)</td>
<td>133 ± 4.2†</td>
<td>388 ± 5.9*</td>
<td>12.5 ± 2.7†</td>
<td>492 ± 40†</td>
<td>0.54 ± 0.21</td>
</tr>
<tr>
<td>BOR (4)</td>
<td>96 ± 5</td>
<td>345 ± 9.5</td>
<td>5.6 ± 1.6</td>
<td>257 ± 8</td>
<td>0.35 ± 0.38</td>
</tr>
<tr>
<td>OHR (5)</td>
<td>122 ± 8.3†</td>
<td>370 ± 7.7</td>
<td>7.4 ± 2.2</td>
<td>539 ± 41†</td>
<td>4.8 ± 1.7‡</td>
</tr>
<tr>
<td>OHR + captopril (5)</td>
<td>99 ± 4.7</td>
<td>360 ± 8.9</td>
<td>7.0 ± 1.3</td>
<td>370 ± 52</td>
<td>4.2 ± 0.2†</td>
</tr>
<tr>
<td>SD + vehicle (4)</td>
<td>97 ± 0.7</td>
<td>355 ± 9.5</td>
<td>5.8 ± 0.7</td>
<td>240 ± 18</td>
<td>0.47 ± 0.22</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. Values in parentheses indicate number of animals. Animals were studied at 3 mo of age. *$P < 0.05$ vs. SD + vehicle, OHR + captopril, and BOR. †$P < 0.01$ vs. BOR, captopril and SD + vehicle. ‡$P < 0.001$ vs. BOS, BOR, and SD + vehicle. $t_{P}$, time constant of decay of posttetanic potentiation; $t_{L}$, time constant of decay of long-term potentiation; BOS, Baltimore ouabain-sensitive rats; BOR, Baltimore ouabain-resistant rats; OHR, ouabain-induced hypertensive SD rats.
acquired and inherited forms of ouabain-dependent hypertension.

Absolute values for \( t_p \) and \( t_L \) in the study groups are shown in Table 2. The \( t_p \) values for the preparations from BOS rats tended to be larger and more variable than those measured in other groups, although there was no significant difference in the time course of decay for the PTP phase of potentiation among these groups of rats. In contrast, the \( t_L \) values derived from the recordings of BOS and OHR ganglia were significantly greater than those obtained from BOR, captopril-treated OHR, or SD vehicle rats.

**Relationship between blood pressure and synaptic efficacy.** Among the treatment groups, MAP ranged between 93 and 145 mmHg. When the decay time constants for PTP (\( t_p \)) and LTP (\( t_L \)) were plotted (Fig. 4) as a function of MAP using the data from 15 ganglia derived from BOS and BOR, significant correlations were found for \( t_L \) (\( r = 0.91, P < 0.0001 \)) and \( t_p \) (\( r = 0.61, P = 0.02 \)) vs. MAP. Also shown in this figure are data from a group of OHR before and following treatment with captopril. The slope of the relationship in the outbred rats is similar to that between MAP and \( t_L \) for inbred rats. Although we did not test whether \( t_L \) could be altered in ganglia from normotensive animals treated with chronic captopril treatment, acute application of this antihypertensive agent did not affect \( t_L \) (\( t_L = 256 \pm 1.8 \text{ min}, n = 5 \)). This value for \( t_L \) was similar (\( P < 0.005 \)) to that observed in the ganglia from SD vehicle rats (Table 2).

We also tested whether the enhanced \( t_L \) seen in BOS and OHR was better related to blood pressure-induced changes in the number of presynaptic fibers firing as opposed to an enhancement of synaptic efficacy of the postganglionic fibers. We therefore plotted the absolute magnitude of pretetanic CAP and peak ganglionic potentiation (\( I_{\text{max}} \)) of all treatment groups as a function of MAP and, as shown in Fig. 5, no correlation was observed for PTP. Similarly, no significant correlation was found when LTP was considered (\( r = 0.142, n = 25 \)). These results suggest that a parameter of synaptic plasticity (\( t_L \)) is linked specifically with blood pressure in our hypertensive animals.

**Spike frequency accommodation in the SCG of OHR.** Postsynaptic activity was assessed by examining spike frequency accommodation in the SCG (46). Figure 6 shows the relationship between the intensity of the depolarizing current and the number of action potentials recorded from the neurons in the SCG from SD vehicle rats and OHR. The neurons in ganglia from the SD vehicle rats invariably generated a single action potential with threshold to four times threshold currents. In contrast, as the current was increased beyond threshold, all the neurons studied in the ganglia from the OHR generated multiple numbers of action potentials. The resting membrane potentials and membrane input resistances were not significantly different (\( P < 0.05 \)) between the neurons from the SCG of SD vehicle rats vs. OHR.

**DISCUSSION**

The principal results from this study are threefold. First, both acute and long-term infusions of ouabain or digoxin depress a short-term component of synaptic plasticity (PTP) in the rat SCG to a comparable extent. As prolonged infusions of ouabain but not digoxin raised blood pressure, the similar effect of these steroids on PTP appears to be unrelated to their distinct effects on long-term blood pressure. Second, in the SCG of rats given long-term infusions of ouabain, there was a significant prolongation in the time constant describing the decay of LTP, indicating that the overall efficacy of sympathetic synaptic transmission was enhanced. The prolongation of LTP was dependent on the sustained elevation of plasma ouabain, was correlated significantly with blood pressure, and was reversed by the prolonged oral administration of captopril. Third, the duration of LTP was augmented also in rats that had been inbred for seven generations based on heightened sensitivity of blood pressure to infusions of exogenous ouabain. In contrast, LTP was normal in the counter strain that was propagated based on heightened sensitivity of blood pressure to infusions of exogenous ouabain. Taken together, these results therefore provide the first direct evidence of the linkage of altered sympathetic ganglionic function with blood pressure in two models of ouabain-dependent hypertension.

**Fig. 4.** Relationship between blood pressure and the decay time constant of PTP and LTP in the SCG from inbred and outbred rats. Each point represents the decay time constant (\( t \)) for PTP (\( t_p \); ○) or for LTP (\( t_L \); ●) from a single animal and the corresponding MAP. The relationship between \( t_p \) and blood pressure was linearly correlated (\( r = 0.906, P < 0.0001 \), slope = 6.7 min/mmHg and \( r = 0.520, P = 0.047 \), slope = 0.15 min/mmHg, respectively). The dashed lines are the 95th percentile confidence intervals. Data are from isolated ganglia from BOS (\( n = 7 \)), BOR (\( n = 4 \)), and SD vehicle (\( n = 4 \)) rats. ○, Mean ± SE values for the relationship between MAP and \( t_p \) in OHR (\( n = 5 \)) or in OHR treated with captopril (OHR + captopril, \( n = 5 \)).
ouabain-dependent hypertension. In the first model (OHR), the hypertension is acquired secondary to the prolonged administration of exogenous ouabain and elevated circulating concentrations of this steroid (31). In the second model, the hypertension is secondary to a genetically determined increase in the sensitivity to EO, develops spontaneously before 7 wk of age, and is not dependent on elevated circulating levels of EO (Table 2).

In view of the method by which BOS rats were derived and propagated, the similarity of the changes in the SCG in both the acquired and genetic models and the equivalence of their association with sustained hypertension are striking. For example, in the OHR model, the slope of the relationship among $t_L$ and blood pressure was indistinguishable from that obtained using ganglia from the inbred strains. Thus a similar dynamic relationship exists between the measured ganglionic and hemodynamic parameters among the inbred strains and the ouabain-infused outbred rats.

Use-dependent synaptic plasticity: the magnitude of PTP. In the rat, the SCG exhibit two forms of use-dependent synaptic plasticity, namely, PTP and LTP. After a brief presynaptic tetanus, the efficacy of nicotinic synaptic transmission increases for a few minutes (PTP). Subsequently, an additional component of synaptic potentiation is observed, termed LTP, that typically persists for several hours (5). It should be noted that we used extracellular recording (the postganglionic CAP) to access PTP and LTP in sympathetic ganglia. This indirect measure of synaptic efficacy precludes distinguishing any postsynaptic changes contributing to PTP or LTP in the SCG from the hypertensive animals. However, measurements of evoked excitatory postsynaptic potentials with intracellular recording techniques in sympathetic ganglia from normal rats have shown that both PTP and LTP are produced exclusively by presynaptic changes (2, 22). In the following discussion, however, we have assumed that PTP and LTP are caused exclusively by presynaptic changes in the SCG from the hypertensive animals.
In the ganglia from the control SD vehicle rats, PTP was recorded as an approximately eightfold increase in the magnitude of evoked postganglionic responses (Fig. 2). PTP was dramatically reduced (<1-fold increase) in rats given prolonged infusions of either ouabain or digoxin, whereas blood pressure was elevated only in the ouabain-infused animals. In the ouabain-infused rats, the decline in PTP preceded the rise in blood pressure. Collectively, these results show that, in the outbred animals, changes in PTP are not linked with long-term blood pressure levels. PTP is considered to be a reflection of cardiac homeostasis by the presynaptic nerve terminals (22). Thus the similar effects of ouabain and digoxin are consistent with the hypothesis that these steroids inhibit presynaptic sodium pumps in the SCG and raise terminal calcium via sodium/calcium exchange. The altered PTP in the SCG may also have some relevance to the net effects of cardiac glycosides on the baroreflex control of the circulation (39, 40) and the amount of sympathetic traffic reaching the heart. For example, ouabain and digoxin augment the baroreflex control of the circulation (37, 44). Increased discharge of the baroreceptor nerves has been documented, whereas heightened ganglionic function has been suspected also as a component of the overall response (45). Our results demonstrate significant effects of therapeutically relevant doses of ouabain and digoxin on PTP in the SCG. These ganglionic effects would be expected to amplify baroreflex-mediated changes in central sympathetic outflow and thereby contribute to the augmentation in reflex control of the heart and vasculature in response to ouabain and digoxin.

Use-dependent synaptic plasticity: kinetics of PTP and LTP. The ganglia from OHR and BOS rats showed large changes in use-dependent synaptic plasticity. Specifically, \( t_L \) increased markedly, whereas a smaller increase in \( t_P \) was also observed. The absolute magnitudes of LTP or PTP were not significantly different in the BOS and BOR strains. In contrast, the decay kinetics of potentiated synaptic transmission differed between the two inbred strains. For example, \( t_L \) was most highly and, apparently, continuously correlated (Fig. 4) with MAP over the range from 92 to 145 mmHg. We suspect that the increase in \( t_L \) in BOS and OHR is secondary to a long-term increase in preganglionic nerve traffic and that the latter arises from activation of the brain renin-angiotensin system. Recent observations have shown that brain ouabain increases central angiotensinogen expression. Moreover, blockade of brain ouabain prevents the upregulation of angiotensinogen and salt-induced hypertension in Dahl salt-sensitive rats (49), whereas ouabain-induced hypertension is blocked by captopril and antagonists of ANG I receptors (36). Therefore, it is of interest that treatment of the OHR with captopril normalized \( t_L \) and MAP (Fig. 4). Importantly, the plasma levels of ouabain remained elevated in the captopril-treated rats (Table 2). This shows that the long-term effects of ouabain in vivo on the kinetics of LTP are not direct effects of the steroid itself but are mediated by an angiotensin-dependent mechanism. When taken together, the available evidence suggests that the altered kinetics of PTP and LTP in the OHR and BOS are dependent on the enhanced generation of angiotensins.

Magee and Schofield (26) reported that postganglionic CAPs elicited by a train of orthodromic stimuli were greater in amplitude in the SHR compared with normotensive controls. Their data revealed that one form of synaptic plasticity, frequency facilitation, was enhanced in the SHR relative to Wistar-Kyoto rats. Our data show that \( t_L \) another form of synaptic plasticity, is enhanced in rats with both acquired and inherited forms of ouabain-related hypertension. Thus our results agree in principle with those reported for SHR, but they were obtained under conditions that avoid concerns about the normotensive strain used for comparison (46). Nevertheless, the observations that synaptic plasticity in the sympathetic ganglia is enhanced in three distinct models of hypertension (SHR, BOS, OHR) suggest that enhanced ganglionic synaptic transmission may contribute to or be a result of sustained increases of SNA and could be a fundamental feature of many hypertensive states. Additional support for this inference comes from the relationship between LTP and blood pressure in OHR before and during captopril treatment, which paralleled the relationship between those variables in the inbred strains (Fig. 4). Moreover, OHR are neither overtly salt nor diuretic sensitive (29, 33), and the bulk of excess SNA probably may not be subject to arterial baroreceptor control.

In sympathetic ganglia, LTP is manifested by changes in presynaptic properties (5). At least two general hypotheses might explain how changes in preganglionic sympathetic synaptic plasticity occur. First, ouabain may modify LTP indirectly by affecting centrally governed autonomic impulse traffic in the preganglionic nerves. This is supported by studies showing that the increased sympathoexcitation and raised blood pressure evoked by ouabain were blocked by the central administration of either ouabain-binding antibody fragments or by ANG II type 1 receptor antagonists (17–19). Thus ouabain heightens peripheral SNA, although it is not known whether this effect was due to an increase in preganglionic nerve activity. A second possibility is that ouabain induces a modification of LTP via other mechanisms. In the SCG, LTP can be induced by the release of an unidentified pre-synaptic peptide factor (5). In addition, the rat SCG contain a number of small intensely fluorescent cells that contain 5-HT. Selective activation of 5-HT3 receptors can induce and maintain LTP (1). The release and/or half-life of one or more of these factors might be affected by in vivo ouabain. Finally, although our work reveals an apparently tight linkage between LTP and blood pressure, with no obvious steady-state hysteresis, our results do not directly exclude long-term changes in MAP itself as the stimulus that modifies LTP. However, the time course of LTP was not altered in the SCG of (mREN-2/27 hypertensive transgenic rats that express an exogenous renin gene and in
plasticity within the sympathetic cervical ganglia and ouabain in the rat evoke enhanced long-term synaptic potentiation. Further investigation of the interactions of ouabain and EO with the nervous system is warranted. In summary, prolonged increases of circulating ouabain in the rat evoke enhanced long-term synaptic plasticity within the sympathetic cervical ganglia and sustained elevations of blood pressure. These phenomena are ouabain specific, reversed by captopril, are inheritable traits, and appear to be tightly linked. Our work suggests that altered synaptic plasticity along with changes in postsynaptic properties of neurons in the SCG may contribute to the induction and maintenance of high blood pressure in conditions where elevated circulating levels of ouabain or EO have been described.

The authors thank K. Moore and G. Taylor for constructive suggestions on an early draft of this manuscript as well as expert technical assistance. We thank Dr. D. Diz (Hypertension Center, Wake Forest University) for help with transgenic rats.

This work was supported by National Institutes of Health Grant Neuroscience Training Grant NS-07375 (A. Aileru), in part by the American-Italian Society of Nephrologists (P. Manunta), American Heart Association (J. M. Hamlyn), Research Infrastructure in Minority Inst. Grant RB-11583 (A. Aileru), and Minority Biomedical Research Support Supplement Grant S06GM 08040 (A. A. Aileru).

Present address of A. A. Aileru: Department of Life Sciences, Winston Salem State University, 601 Martin Luther King Blvd., Winston Salem, NC 27110.

Present address of A. de Albuquerque: Departamento de Fisiología e Farmacología, Universidade Federal do Ceará, Fortaleza, 60430-270 CE, Brazil.

Present address of P. Manunta: Division of Nephrology, University of Milan, San Rafael Hospital, Via Olgettina 60, 20132 Milan, Italy.

REFERENCES


