Inhibition of Ganglionic Long-Term Potentiation Decreases Blood Pressure in Spontaneously Hypertensive Rats

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Long-term potentiation of sympathetic ganglia (gLTP), a unique form of synaptic plasticity, is serotonin dependent and can be blocked with 5-HT_3 receptor antagonists. Long-lasting enhancement of the basal tone of ganglionic transmission (as with gLTP) is expected to result in sustained increase in peripheral resistance that would lead to elevated blood pressure. We examined the possibility that in sympathetic ganglia, gLTP may be involved in the expression of stress-induced (neurogenic) form of hypertension. High blood pressure in spontaneously hypertensive rat (SHR), known to show exaggerated cardiovascular defense reactions to environmental stimuli, is partly due to a neurogenic factor. Chronic treatment of SHR and their normotensive counterpart, the Wistar Kyoto (WKY) rats with the 5-HT_3 receptor antagonist tropisetron (ICS; 5 mg/kg/day), caused a marked decrease in the blood pressure of the SHR but not of WKY rats. Increasing the daily dose of ICS cumulatively (7 and 10 mg/kg) did not result in significant additional decrease in blood pressure of SHR, indicating that the drug blocks only the neurogenic component of hypertension in the SHR. electrophysiological procedures for indirectly testing for the presence of gLTP in ganglia excised from SHR suggest that gLTP has been previously expressed in these ganglia *in vivo*. This contrasts with the absence of gLTP in ganglia from normotensive rats. The results support contribution of gLTP to the expression of neurogenic hypertension.

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In mammalian sympathetic ganglia, long-term potentiation (LTP) has been demonstrated both *in vivo* (1–3) and *in vitro* (4–8). In these ganglia, a brief high frequency tetanic stimulation (tetanus) of the preganglionic nerve induces LTP in the form of a long-lasting enhancement of the nicotinic pathway. Expression of this activity-dependent LTP in the superior cervical ganglion (SCG) of the rat produces no change in the acetylcholine content of the ganglion (5), or in the postsynaptic membrane sensitivity to applied nicotinic agonists (9). Similar results have been reported for the LTP of the avian parasympathetic ciliary ganglion (10). Ganglionic LTP (gLTP) is independent of the activation of cholinergic, adrenergic (5), or adenosine receptor (11), and is due to an increase in acetylcholine release as measured by biochemical assay (5). However, in addition to tetanus, induction of gLTP requires activation of 5-HT_3 receptors by serotonin, presumed to be released from small intensely fluorescent (SIF) cells within the SCG of the rat (8). Activation of the 5-HT_3 receptor is, surprisingly, also required for the maintenance phase of gLTP. This has been demonstrated by the sensitivity of gLTP to block by 5-HT_3 antagonists (8). Other serotonin receptor subtypes do not seem to be involved in this response (8). Furthermore, there is evidence for the involvement of carbon monoxide in the induction phase (12) and nitric oxide in the maintenance phase of gLTP (13, 14).

Hyperactivity of the sympathetic cardiovascular control is believed to contribute to many types of hypertension in patients. It is well known that genetic factors also contribute to the development of hypertension, and often there is interplay of various factors. In humans, mental stress produces a greater rise in blood pressure in patients with labile hypertension than in normotensive subjects (15, 16). Although stress-induced hypertension is known to normalize shortly after stress is removed, prolonged mild-moderate hypertension may contribute significantly to atherosclerotic cardiovascular diseases (17). A process such as gLTP that produces a sustained enhanced activity of the sympathetic nervous system may well be responsible for the develop-

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ment and/or aggravation of certain forms of hypertension. Thus, we hypothesize that a sustained increase in central sympathetic outflow (as with mental stress) to ganglia may provide the repeated high frequency presynaptic activity required for expression of gLTP in sympathetic ganglia. This would result in a sustained increase in sympathetic tone to the blood vessels, leading to hypertension. We tested this hypothesis in the spontaneously hypertensive rat (SHR), which is known to have exaggerated cardiovascular responses to external stressful stimuli (18, 19).

Materials and Methods

Electrophysiological Recording of gLTP in Isolated Ganglia. All procedures involving animals were carried out in accordance with NIH's *Guide for the Care and Use of Laboratory Animals* and with the guidelines of the University of Houston Institutional Animal Care and Use Committee. Ganglia were rapidly excised and carefully desheathed in oxygenated (95% O_2, 5% CO_2) Locke solution (pH 7.4) containing (in millimoles): NaCl 136, KCl 5.6, $CaCl_2$ 2.2, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 16, glucose 11, and 0.02 choline chloride. For recording postganglionic compound action potentials (CAPs), the ganglion was placed in a constant temperature (32° ± 1°C) chamber (3 ml), and the preganglionic (cervical sympathetic) and postganglionic (internal carotid) nerves were gently drawn into capillary-stimulating and recording suction electrodes, respectively. The ganglion was continuously superfused with Locke solution at a rate of 1.5 ml/min. The CAPs were evoked by supramaximal stimulation of the preganglionic nerve by square wave pulses (duration of 0.3 msec) at a rate of 0.017 Hz. The CAPs were amplified and displayed on a digital storage oscilloscope. The digitized CAPs were plotted on paper (Astro-Med 102XLA oscillograph). After stabilization, hexamethonium (0.3–0.4 mM in Locke solution) was used to partially block the nicotinic pathway in order to obtain submaximal CAPs for better detection of gLTP (14). This concentration of hexamethonium produces approximately 50% reduction in the amplitude of the CAP. An additional period of stabilization of 1 hr at the new sub-

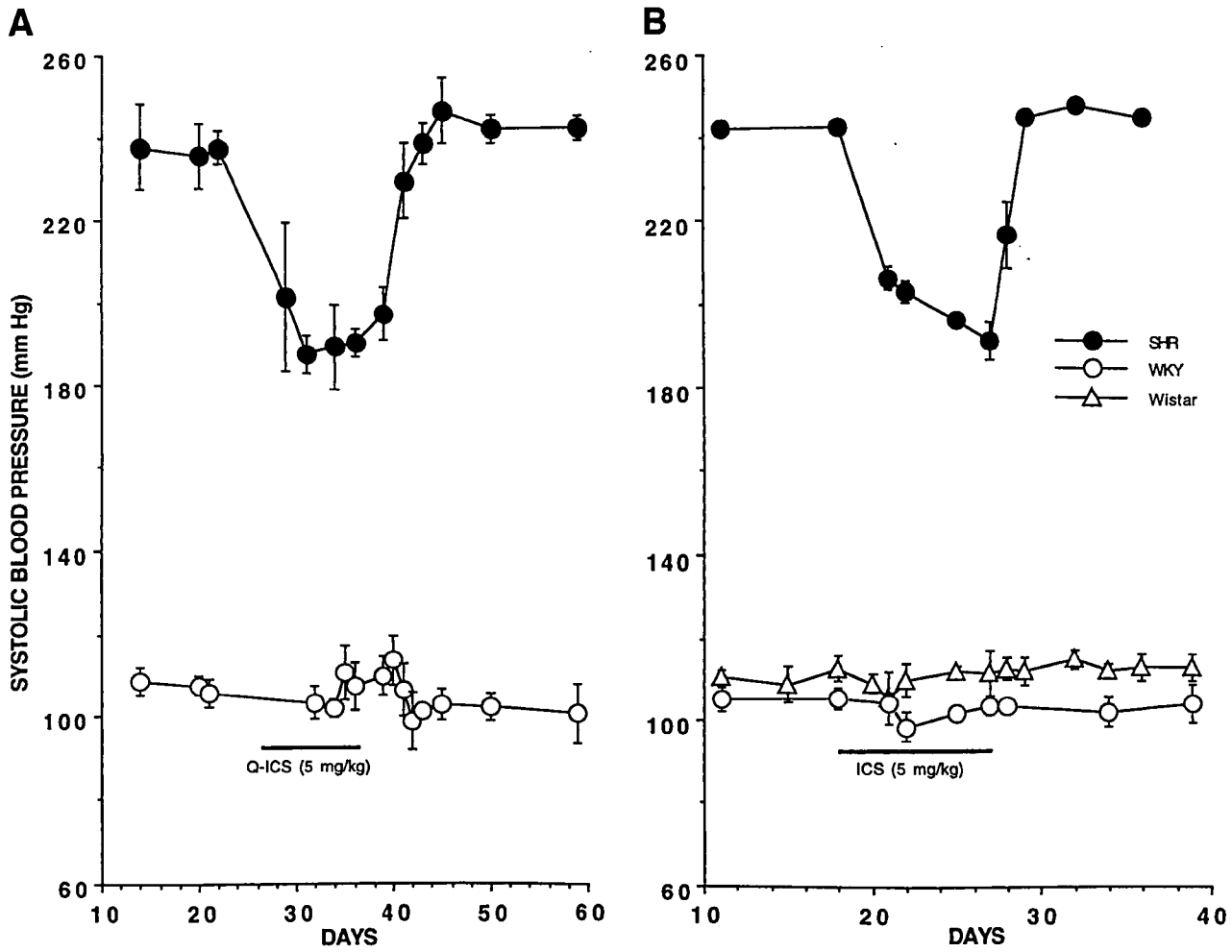

Figure 1. Chronic treatment of SHR and WKY rats with tropisetron (ICS). (A) Quaternary form of the 5-HT_3 receptor antagonist tropisetron (Q-ICS, 25 mg/kg, i.p. twice daily for 10 days, black bar) results in a marked decrease in blood pressure of the SHR (•) but not WKY (○). (B) Similar results were obtained using the tertiary form of ICS with Wistar rats (△) as a second control. Blood pressure was measured by the tail-cuff method. Each point in each series is the mean ± SD from five SHR, six WKY, or four Wistar rats.

maximal amplitude was allowed. Following this, a tetanizing pulse (20 Hz for 20 sec) was applied to the preganglionic nerve. After tetanus, test pulses were resumed and recordings made every 2 min for the first 10 min, then every 5 min for the first hour, and finally every 10 min for the next 2 hr. Changes in amplitude of CAP were expressed as percentage of that of CAP spike recorded immediately before tetanic stimulation.

Measurement of Blood Pressure. Systolic blood pressure was measured by the indirect tail-cuff plethysmography from 18 SHR and 17 Wistar Kyoto (WKY) (age 12 weeks). This method has been shown to be as accurate as the direct method (artery catheterization) when both measurements were made repeatedly and simultaneously (20). Tail cuff, 1.5 cm in diameter and 3.2 cm in length, was used. Systolic pulsation was detected by an electrosphygmograph coupler (NarcoBioSystem, Houston, TX), and the pressure and pulsation were transduced by a pneumatic transducer and recorded on a physiograph. Rats were placed in clear acrylic holders where they were slightly warmed to induce vasodilatation until pulsation can be recorded. Each animal was acclimated to being in the holder and to the sensation of the cuff inflation-deflation cycles for 7–10 days prior to obtaining a stable blood pressure baseline. Systolic blood pressure was indicated by the onset of pulsation where it was averaged from 4–8 measurements for each rat.

Because SHR and WKY rats are genetically closely related, we included "normal" rats (Wistar or Sprague Dawley) as controls in some blood pressure measurements and electrophysiological series. This is to control for the possibility that differences between SHR and WKY could be the result of WKY being different from the normal rat population.

Drugs. Quaternary and tertiary tropisetron and MDL 72222 were purchased from Research Biochemicals International (RBI). Stock solutions were made fresh daily in normal saline, and doses contained in 0.3–0.5 ml were injected i.p.

Statistical Analysis. In comparing values under different conditions, test of significance was made by using the paired t test, unpaired t test, or analysis of variance as appropriate using GB-Stat 6.5.2 computer program (Dynamic Microsystems, Silver Spring, MD); P values of 0.05 or less were considered significant.

Results

Chronic Treatment with Tropisetron. We hypothesize that gLTP contributes to the elevated blood pressure in hypertensive animals. If this is the case, then blocking gLTP in these animals should result in a decrease in the blood pressure. We examined this hypothesis using the genetic animal model of hypertension, the SHR and its normotensive counterpart, the WKY as control. Blood pressure in both groups was measured over a period of 10 days to establish a baseline, then rats in both groups were injected (2.5 mg/kg, i.p.) twice daily with the quaternary form of the selective serotonin 5-HT_3 receptor antagonist, tropisetron (Q-ICS), for the next 10 days. Systolic blood pressure for both groups was monitored every 2–3 days during the period of treatment. During this treatment, we measured a significant ($P < 0.01$; $n = 5$) decrease in the blood pressure of SHR but not in WKY (Fig. 1A). Termination of the treatment resulted in return of the blood pressure to the pretreatment level in 4–6 days. Similar results were obtained in a second series of experiments when the tertiary form of the drug was used with an additional group of Wistar rats as a second control (Fig. 1B).

To determine whether high concentrations of ICS can normalize blood pressure in SHR, we examined the effect of increasing cumulative doses of ICS on blood pressure of SHR and WKY. Daily treatment of SHR with 5 mg/kg ICS produced significant ($P < 0.01$) decrease in blood that was maintained for 10 days. When the daily dose was increased to 7 mg/kg over the following period of 10 days, it produced no significant additional decrease of blood pressure. The daily dose of ICS was again increased to 10 mg/kg and treatment continued over the following next 10 days. During that period, the SHR blood pressure remained not significantly different from the level reached during the 7 mg/kg treatment period and slightly but significantly ($P < 0.05$) lower than that at the 5 mg/kg level (Fig. 2). The WKY blood pressure was not significantly affected by similar treatment of these rats with the same doses of ICS (Fig. 2).

Evidence for the Presence of gLTP in Ganglia Excised from Hypertensive Rats. We hypothesize that transmission in sympathetic ganglia from hypertensive animals has already been potentiated due to *in vivo*-expressed gLTP. We tested this hypothesis in two series of experiments.

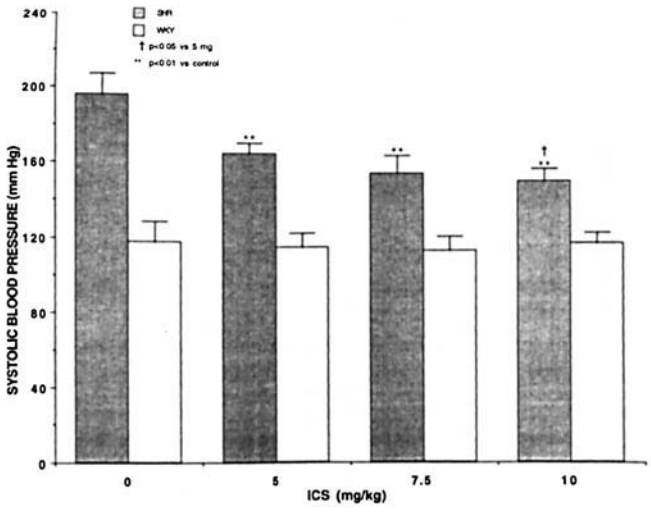

Figure 2. Tropisetron (ICS) blocked only a neurogenic component of hypertension in SHR. Daily treatment with increasing, cumulative doses of ICS caused only a small additional reduction of the blood pressure of SHR. The same doses had no significant effect on blood pressure of the normotensive WKY. Blood pressure was measured by the tail-cuff method. Each bar in each series represents mean systolic blood pressure (± SD) from five SHR or six WKY rats measured 10 days after a particular dose.

If ganglionic transmission is potentiated as a result of expression of gLTP *in vivo*, then bath application of a 5-HT_3 receptor antagonist should block gLTP and thus decrease CAP amplitude of naïve (unstimulated) ganglia from hypertensive animals without affecting that of ganglia from normotensive animals. Ganglia excised from SHR, WKY, and Wistar rats were setup for electrophysiological recording of CAP as an index of ganglionic transmission. After stabilization of baseline CAP, the 5-HT_3 receptor antagonist, ICS (0.5 μM) or MDL 72222 (0.5 μM) was superfused on ganglia. MDL 72222 markedly decreased the amplitude of CAP spike of ganglia from SHR without significantly affecting that of ganglia from WKY or Wistar rats (Fig. 3). Similar results were obtained with ICS. One hour after superfusion of ICS, the amplitude of CAP from WKY was 97.5% ± 2.5% of baseline CAP (n = 6); Wistar; 94.3% ± 4% (n = 4) and SHR; 78% ± 2.5% (n = 7).

Like all forms of LTP, once gLTP is induced, a second tetanus does not significantly influence the already enhanced transmission (Fig. 4, arrowhead 2). Because of this saturability, if gLTP has already been expressed *in vivo* in sympathetic ganglia from hypertensive animals, then no additional enhancement of transmission can be induced in these ganglia by tetanus *in vitro*. When ganglia from SHR, WKY, and Sprague Dawley (SD) rats were subjected to tetanus (20 Hz/20 sec), those from WKY (n = 11) and SD rats (n = 5) expressed a well-maintained, robust gLTP (Fig. 4). Similar tetanus produced small or in some cases, no potentiation in ganglia from SHR (Fig. 4; n = 15), suggesting that gLTP has been already expressed in ganglia from these hypertensive animals.

Discussion

An obvious outcome from the expression of gLTP in sympathetic ganglia is an increase in sympathetic outflow to the cardiovascular system. A possible cause for induction and expression of gLTP *in vivo* is an increased central sympathetic discharge that would mimic the tetanus required for inducing gLTP *in vitro*. Thus, repeated stressful mental stimuli *in vivo* may lead to a situation much like the experi-

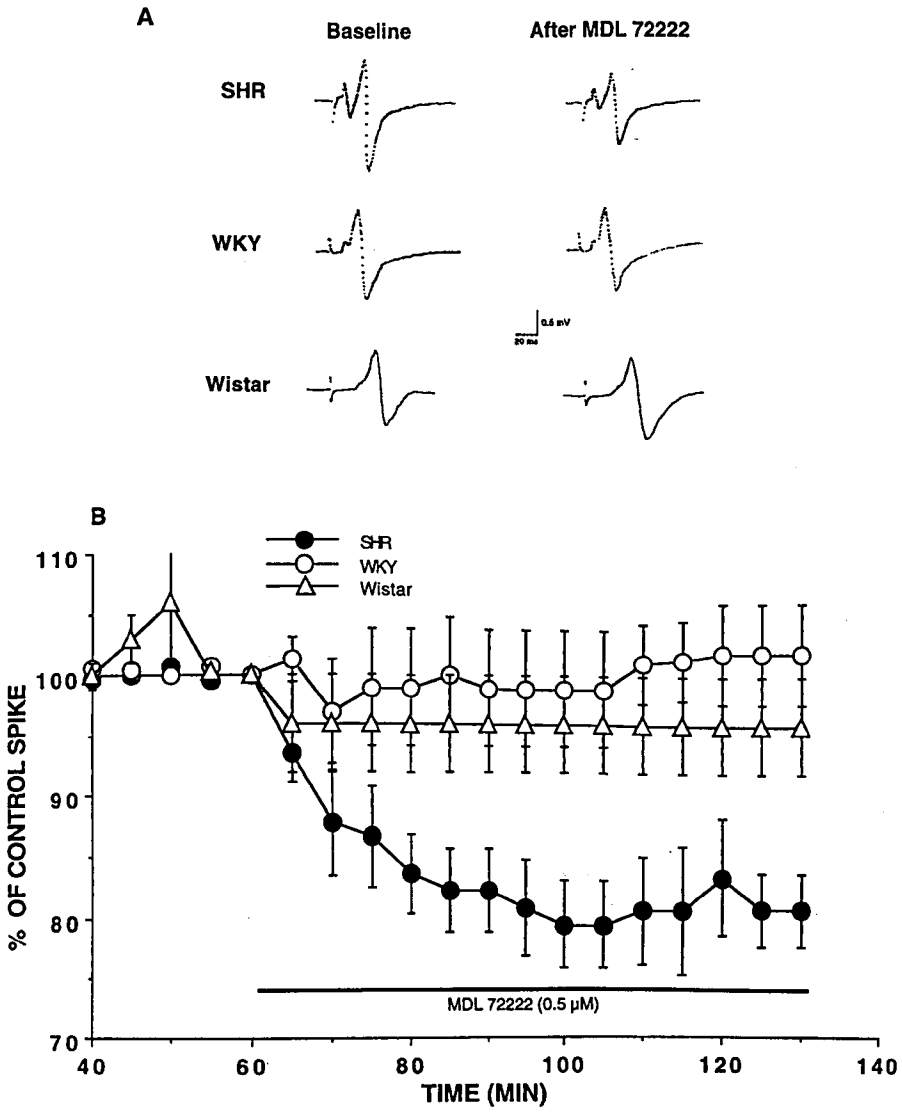

Figure 3. Inhibition of baseline ganglionic transmission by a 5-HT_3 receptor antagonist. (A) Representative experiment in which the 5-HT_3 receptor antagonist, MDL 72222, decreased baseline CAP of ganglia isolated from SHR, but not of those isolated from WKY or Wistar rats. Calibrations apply to records of all groups. (B) Summary of all experiments where MDL 72222 (0.5 μM, black horizontal bar), decreased baseline CAP of ganglia isolated from SHR (•), but not in those isolated from WKY (○) or Wistar (△) rats. Each point in each series is the mean ± SEM from five to seven ganglia.

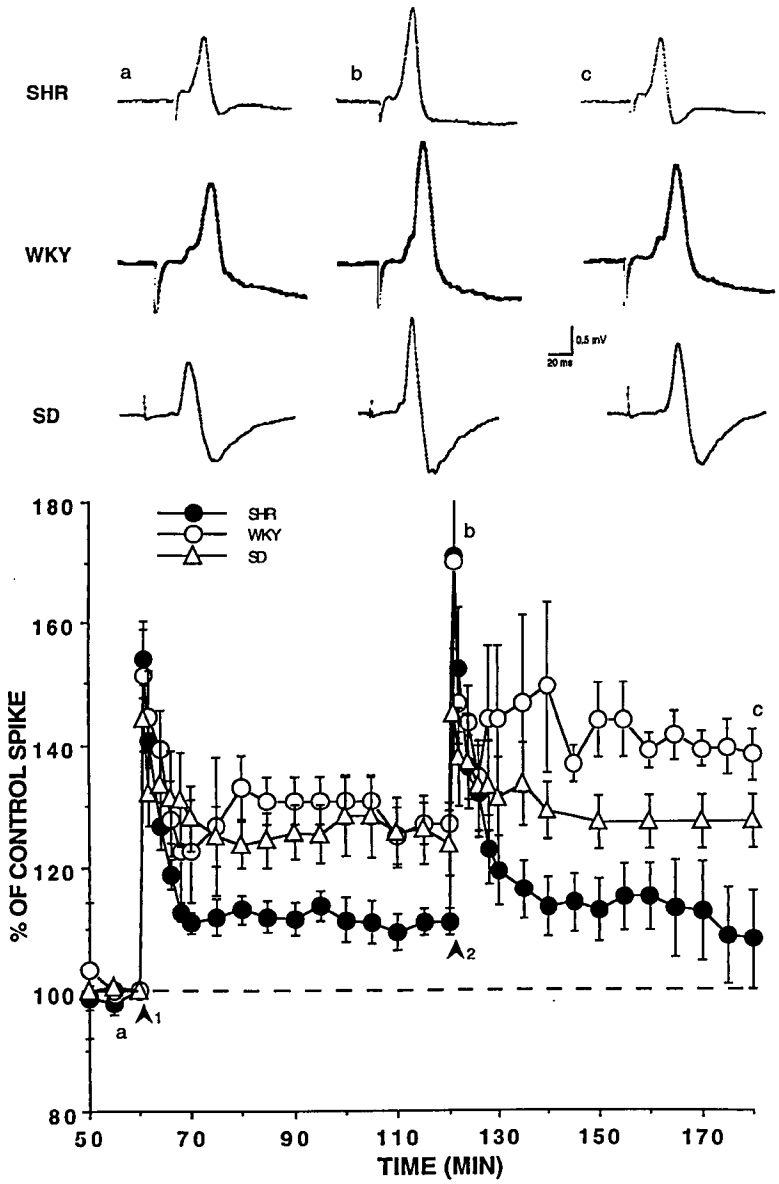

Figure 4. Tetanus (20 Hz/20 sec, arrowhead 1) of the preganglionic nerves evoked robust gLTP in ganglia excised from WKY (○) or Sprague Dawley (SD, △) rats. A second volley of tetanus (arrowhead 2) failed to produce significant additional potentiation of ganglionic transmission due to saturation. In a similar series of experiments using identical protocol, tetanus of preganglionic nerves of ganglia excised from SHR (•) produced very small enhancement of transmission. Similarly, in this series, a second tetanus failed to evoke gLTP. Each point represents the mean ± SEM from 5 SD, 11 WKY, and 15 SHR ganglia. Inset shows records of CAP of ganglia from SHR, WKY, and SD taken at times indicated on graph.

mental tetanus that generates gLTP in ganglia *in vitro*. This sustained enhancement of synaptic efficacy would lead to a substantial increase in sympathetic tone and a consequent increase in peripheral resistance that is manifested as hypertension. Evidence indicates that one of the major factors in the development of hypertension in the SHR and borderline hypertensive rats is increased sympathetic nerve activity (21, 22). The SHR is also known to show exaggerated cardiovascular defense reactions to environmental stresses (18, 19). Experiments involving social isolation of SHR after weaning emphasize the importance of the interactions between extrinsic-hereditary and extrinsic-neurogenic factors for initiation of hypertension. The socially isolated SHR developed lower arterial pressure than those kept in groups (23). Similar findings were reported in studies on humans (see Ref. 24). Additional evidence for a neurogenic contribution to hypertension in SHR is the observation that ganglionic blockade lowers blood pressure to a greater degree in SHR than in renal hypertensive rats with comparable blood pressure (25). Although a greater vascular reactivity to neurotransmitter could contribute to this difference between SHR and the renal hypertensive rats, an increase in sympathetic tone via enhanced ganglionic transmission is an equally possible contribution to the hypertension in SHR.

In the present study, we show indirect evidence suggesting that ganglionic transmission is enhanced by a mechanism sensitive to inhibition by a 5-HT_3 receptor antagonist only in the SHR. Intracellular recording techniques demonstrated that synaptic transmission was enhanced in the SCG of adult (hypertensive) SHR, but not in those of the normotensive young SHR, WKY, or Wistar rat (29). Comparing the EPSP and EPSC of postganglionic neurons from hypertensive (adult SHR) and normotensive (young SHR, WKY, and Wistar) rats, Magee and Schofield (29) showed that they are similar in all electrophysiological characteris-

tics except that in the hypertensive adult SHR these responses have significantly larger amplitude. The larger amplitude was most likely due to enhanced presynaptic release of acetylcholine, as indicated by elevation of quantal content of the EPSP/EPSC of adult SHR neurons (29).

We present evidence that suggests a link between the development of stress-induced hypertension and the expression of gLTP in sympathetic ganglia *in vivo*. The decrease in the blood pressure of SHR during treatment with Q-ICS is most probably due to blockade of the 5-HT_3 receptors in sympathetic ganglia because it is unlikely that the quaternary form of the drug could cross the blood-brain barrier to act centrally. However, it could be argued that the quaternary form of ICS might still access the brain at the circumventricular organs, particularly the area postrema, to produce reduction in the blood pressure. This is unlikely even though the area postrema is known to have both high densities of 5-HT_3 receptor binding sites and a strong association with the nucleus tractus solitarius of the medulla oblongata that contains the cardiovascular control centers (26). Because the 5-HT system in the nucleus tractus solitarius is not different in the SHR and WKY (30), it is improbable that the drug, acting at the area postrema, markedly decreases blood pressure in the SHR without affecting that of the WKY. Furthermore, both the quaternary form and the tertiary form produced nearly identical effect on blood pressure of the SHR. It could still be argued that the drug produces its hypotensive effect by acting on a central site only when there is hypertension. However, results from dose-response series indicated that higher doses of the 5-HT_3 receptor antagonist produced only a minimal further drop in the still markedly elevated blood pressure of the SHR. As our hypothesis would predict, the 5-HT_3 receptor antagonist blocked only the neurogenic component (caused by gLTP) of the hypertension.

Alternatively, the blocking effect of 5-HT_3 antagonists on potentiated synaptic transmission might be due to a mechanism other than specific inhibition of gLTP. A high concentration of ICS was reported to antagonize 5-HT_4 receptors as well (27). However, this is unlikely in the present experiments because we used a low concentration of ICS, and we also used another 5-HT_3 antagonist, MDL 72222, with similar results. It has been reported that ICS decreases nicotine-induced increases in locomotor activity (28). If this effect was due to a peripheral nicotinic receptor antagonistic action of ICS, it was not seen in our experiments because the drug had no effect on baseline ganglionic transmission in ganglia from normotensive animals (see Fig. 2).

In conclusion, we demonstrate that 5-HT_3 receptor antagonists, which are known to inhibit gLTP *in vitro*, decrease blood pressure in the SHR but not in normotensive rats. Our results also show that ganglia isolated from SHR seem to have expressed gLTP *in vivo*, whereas those isolated from normotensive rats have not. Together, these results suggest a possible link between development of neurogenic hypertension and expression of gLTP in sympathetic ganglia.

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