Hemodynamic consequences of chronic parasympathetic blockade with a peripheral muscarinic antagonist

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Whereas the sympathetic nervous system has a well-established role in blood pressure (BP) regulation, it is not clear whether long-term levels of BP are affected by parasympathetic function or dysfunction. We tested the hypothesis that chronic blockade of the parasympathetic nervous system has sustained effects on BP, heart rate (HR), and BP variability (BPV). Sprague-Dawley rats were instrumented for monitoring of BP 22-h per day by telemetry and housed in metabolic cages. After the rats healed from surgery and a baseline control period, scopolamine methyl bromide (SMB), a peripheral muscarinic antagonist, was infused intravenously for 12 days. This was followed by a 10-day recovery period. SMB induced a rapid increase in mean BP from 98 ± 2 mmHg to a peak value of 108 ± 2 mmHg on *day 2* of the SMB infusion and then stabilized at a plateau value of +3 ± 1 mmHg above control ($P < 0.05$). After cessation of the infusion, the mean BP fell by 6 ± 1 mmHg. There was an immediate elevation in HR that remained significantly above control on the last day of SMB infusion. SMB also induced a decrease in short-term (within 30-min periods) HR variability and an increase in both short-term and long-term (between 30-min periods) BPV. The data suggest that chronic peripheral muscarinic blockade leads to modest, but sustained, increases in BP, HR, and BPV, which are known risk factors for cardiovascular morbidity.

blood pressure; autonomic nervous system; parasympathetic nervous system; blood pressure variability

THE AUTONOMIC NERVOUS SYSTEM maintains cardiovascular homeostasis through the opposing effects of its parasympathetic and sympathetic divisions on cardiac performance and through a predominantly sympathetic control of the vasculature. Whereas a sympathetic effect on blood pressure (BP) regulation is well established (6), there is also evidence of an involvement of the parasympathetic nervous system in hypertension. This was first suggested by Julius et al. (12) on the basis of acute pharmacological experiments in young individuals with borderline hypertension and a hyperkinetic circulation. Subsequent studies have shown an attenuation of the parasympathetic control of heart rate (HR) in hypertension (33) and in a number of conditions predisposing to hypertension such as ageing (27), obesity (1, 31), diabetes mellitus (3), chronic renal failure (2), and physical inactivity (24). However, it is not clear whether parasympathetic dysfunction may affect basal BP levels or exacerbate the hypertensive state. In addition, since vagal dysfunction impairs the baroreflex control of HR, parasympathetic dysfunction could promote an increase in BP variability (BPV) (7), which has been shown to be an independent cardiovascular risk factor (9).

The purpose of the present study was to test the hypothesis that sustained blockade of the parasympathetic system would lead to increased BP and BPV. To this end, we investigated the effects of a 12-day administration of scopolamine methyl bromide (SMB) in rats instrumented for monitoring of BP by telemetry. SMB, a quaternary derivative of scopolamine, was chosen because it is a muscarinic antagonist that does not readily cross the blood-brain barrier (4) and thus avoids potential confounding effects of central muscarinic antagonism such as increased parasympathetic tone (18).

METHODS

Animal preparation. Male Sprague-Dawley rats (10–12 wk old, weighing ~390–400 g) supplied by the R. Janvier Center (France) were used in this study. All protocols were approved by the State Animal Committee. The rats were instrumented (under pentobarbital anesthesia, 60 mg/kg ip) with a BP telemeter (model TA11-PA-C40, Data Sciences International) for monitoring of aortic BP as described previously (32). A catheter was also inserted in the left jugular vein and connected to a syringe pump via a single channel swivel as described previously (35), for continuous intravenous infusion of isotonic saline (10 μl/min) starting on the day of surgery and maintained throughout the experimental study. The rats were housed in individual Plexiglas cages equipped for 24-h urine collection. The cages were in a quiet air-conditioned room (~21°C) with a 12-h:12-h light-dark cycle.

Continuous hemodynamic monitoring by telemetry. Each cage was equipped with one RMC-1 receiver connected to a calibrated analog adapter (R11CPA) and to an APR-1 barometric pressure reference device. All devices were manufactured by Data Sciences International. The telemetered analog pressure signal was sampled at 500 Hz for 5-s periods every 30 s, 24 h a day and processed by customized algorithms for beat-to-beat analysis to extract mean arterial pressure (MAP), systolic and diastolic blood pressures (SBP, DBP, respectively), HR, rise time (time to reach peak SBP from the previous DBP point), and maximum rate of change of arterial pressure (dP_a/dt) (17). Telemetry probes were calibrated before implantation and after removal in a sealed pressure chamber at 0 and 200 mmHg, and pressure values were linearly corrected according to calibration values (29). Data were analyzed from 10:00 AM to 8:00 AM the next morning. The period between 8:00 AM and 10:00 AM was used for daily maintenance and was omitted from the analysis.

The computer program separated the 22-h data set into a series of 44 half-hour periods. The standard deviation (SD) of BP (for both MAP and SBP) were calculated for each half-hour period. The mean

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SD of BP was used as a measure of the short-term variability of BP, i.e., the extent of the variation in BP within half-hour periods (19, 30). In addition, we also calculated the SD of the 44 half-hourly mean BP values and used the SD as a measure of the long-term variability of BP, i.e., the extent of the variation in BP between half-hour periods (19, 30). Finally, the SD of all 2,640 daily samples was also computed as an index of overall 22 h BPV. Similar calculations were performed to calculate the short- and long-term HR variability. It should be noted that the SD of HR within half-hour periods does not represent beat-to-beat HR variability but the 30s-to-30s variability of HR. It is thus labeled "short-term" HR variability.

Experimental protocol. In all rats, experimental measurements included measurement of BP and HR 22 h a day and monitoring of daily food, water, and sodium intakes, urine output, and sodium and potassium excretions. Food and water intake were determined by differential weighing (precision at 0.1 g). Urine was collected in a graduated container. Urinary sodium and potassium concentrations were determined by flame photometry (model IL 943). All animals had free access to tap water and were fed with a fixed amount of 20 g (slightly below the normal ad libitum intake) of standard rat chow per day to ensure a fixed sodium intake (a total of about 4 mmol/day, including 2.2 mmol/day via the infusion).

In seven rats that were successfully instrumented (10.7 ± 0.2 wk old, weighing 396 ± 14 g), a SMB (Sigma, Switzerland) intravenous infusion was started after at least 12 days after the rats recovered from surgery and an additional control period of 4 days. SMB was infused for 12 days at increasing rates (0.3, 0.6, and 1.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv, 4 days at each dose, labeled as *periods 1*, *2*, and *3*). The progressive increase in the SMB-infusion rate was chosen to ensure sustained muscarinic blockade, since pilot studies in which SMB was infused at the single dose of 0.3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 10 days showed a higher BP during the first 4 days of infusion (approximately +10 mmHg) than during the following days (approximately +5mmHg). The SMB infusion was followed by a 10-day recovery period to test the reversibility of the experimental changes. All solutions (SMB or vehicle saline) were prepared aseptically and infused through a 0.22-μm Millipore filter.

Because BP and HR are known to exhibit a small spontaneous negative drift after surgery, a group of eight rats were submitted to the same surgical procedures (telemetry, intravenous infusion) and housed in identical cages to analyze the changes in BP and HR occurring during vehicle time-control infusion from *day 9* after surgery (when the telemeter battery was turned on) up to 28 days after surgery. In two rats, the arterial BP signal could not be used for computation of MAP due to a large and unstable offset drift but was adequate for the computation of HR. The rats were studied up to 4 wk after surgery to quantify the spontaneous drift of the 22-h average of BP and HR.

Statistical analysis. Each rat served as its own control, i.e., the effects of SMB infusion were compared with the average of the 4 control days preceding SMB infusion. Statistical analysis was performed by repeated measures ANOVA with the InStat statistical package (GraphPad Software). Experimental and control values were compared using Dunnett's multiple comparison test. Changes were considered significant if $P < 0.05$. Values are expressed as means ± SE.

RESULTS

SMB induced a rapid increase in mean, systolic and diastolic pressures, and HR (Fig. 1). MAP peaked on *day 2* of the SMB infusion at +10.5 ± 0.7 mmHg above a control value of 97.9 ± 1.9 mmHg. The initial increase in MAP was attenuated over the next few days but MAP remained significantly elevated at a plateau value of +2.9 ± 0.7 mmHg ($P < 0.05$) during the last 4 days of SMB (*period 3*), as shown in Table 1. On cessation of SMB infusion, MAP fell by 5.9 ± 0.7 mmHg (*day 2* of recovery) and stabilized at 2.6 ± 1.1 mmHg below control value during the last 4 days of the recovery period (*days 7* to *10* after SMB cessation).

HR underwent an immediate increase with SMB infusion, peaking on the first day at +52 ± 5 beats/min above the control value of 345 ± 8 beats/min. HR then tended to decrease progressively but remained significantly above control at +22 ± 5 beats/min by the last day of SMB infusion (*day 12*). After cessation of the SMB infusion, HR fell by 36 ± 4 beats/min (*day 2* of recovery). During the final 4 days of the recovery period, HR reached a value of 318 ± 7 beats/min, i.e., 27 ± 6 beats/min below the control level.

Rats infused with vehicle infusion showed a small decrease in MAP by 3.0 ± 0.9 mmHg from 97.7 ± 1.1 mmHg (average of *days 9–12* postsurgery) to 94.7 ± 1.0 mmHg (average of *days 25–28*), with most of the decrease (2.5 ± 1.1 mmHg) occurring during the first 20 days after surgery. HR showed a larger decrease of 31 ± 5 beats/min from a control value of 361 ± 9 beats/min (average of *days 9–12* postsurgery), with most of the decrease (22 ± 5 beats/min) occurring during the first 20 days after surgery. Subtracting the average daily negative drift of BP and HR observed in time-control rats (using postsurgery *days 13–16* as the control period) from individual values of experimental rats, we estimate the actual effects of SMB on MAP and HR in experimental rats to amount to 10.2 ± 0.4, 5.2 ± 0.7, and 5.5 ± 0.7 mmHg for *periods 1*, *2*, and *3*, respectively, and to 60 ± 5, 53 ± 4, and 47 ± 4 beats/min for the same periods.

Daily values of pulse pressure (PP), maximum dP_a/dt, and rise time in SMB-infused rats are shown in Fig. 2. Changes from control values are summarized in Table 1. PP and maximum dP_a/dt were significantly higher only during the first few days of infusion (on *day 3*, PP peaked at +3.5 ± 0.7 mmHg above the control value of 22.8 ± 1.4 mmHg and maximum dP_a/dt peaked at +840 ± 216 mmHg/s above a control value of 5,062 ± 306 mmHg/s). Rise time was significantly shorter during *days 1–4* (*period 1*) and *5–8* (*period 2*) and tended to remain shorter during the last 4 days of SMB infusion (*period 3*).

Daily values of short-term and long-term MAP and HR variabilities are shown in Fig. 3. SMB induced a progressive increase in both short-term (+16%, last 4 days of SMB infusion) and long-term (+36%) MAP variabilities, which reverted progressively to control values on cessation of the SMB infusion. Interestingly, the changes in long-term variability were slower to appear and also to disappear. The increase in BP variability was significant for both MAP and SBP and for all three indexes of variabilities (within 30 min SD , between 30 min SD, and overall 22 h SD), as shown in Table 2. SMB also lead to a marked (−43%) and sustained decrease in short-term HR variability (HRV). In contrast, the decrease in long-term HRV was only transient (−25% on *day 2*).

Total water intake and urinary output tended to increase progressively during SMB infusion, as shown on Fig. 4 and were significantly higher during the last 4 days of infusion (water intake: + 7.2 ml from a control value of 37.8 ± 1.1 ml/day; urine output: +8.6 ml from control value of 24.7 ± 0.7 ml/day). Both variables returned slowly toward control during the recovery period. Changes occurred in parallel and, as a consequence, daily water balance remained unaltered. In 3 of 7 rats, food intake (fixed at a maximum of 20 g/day) decreased

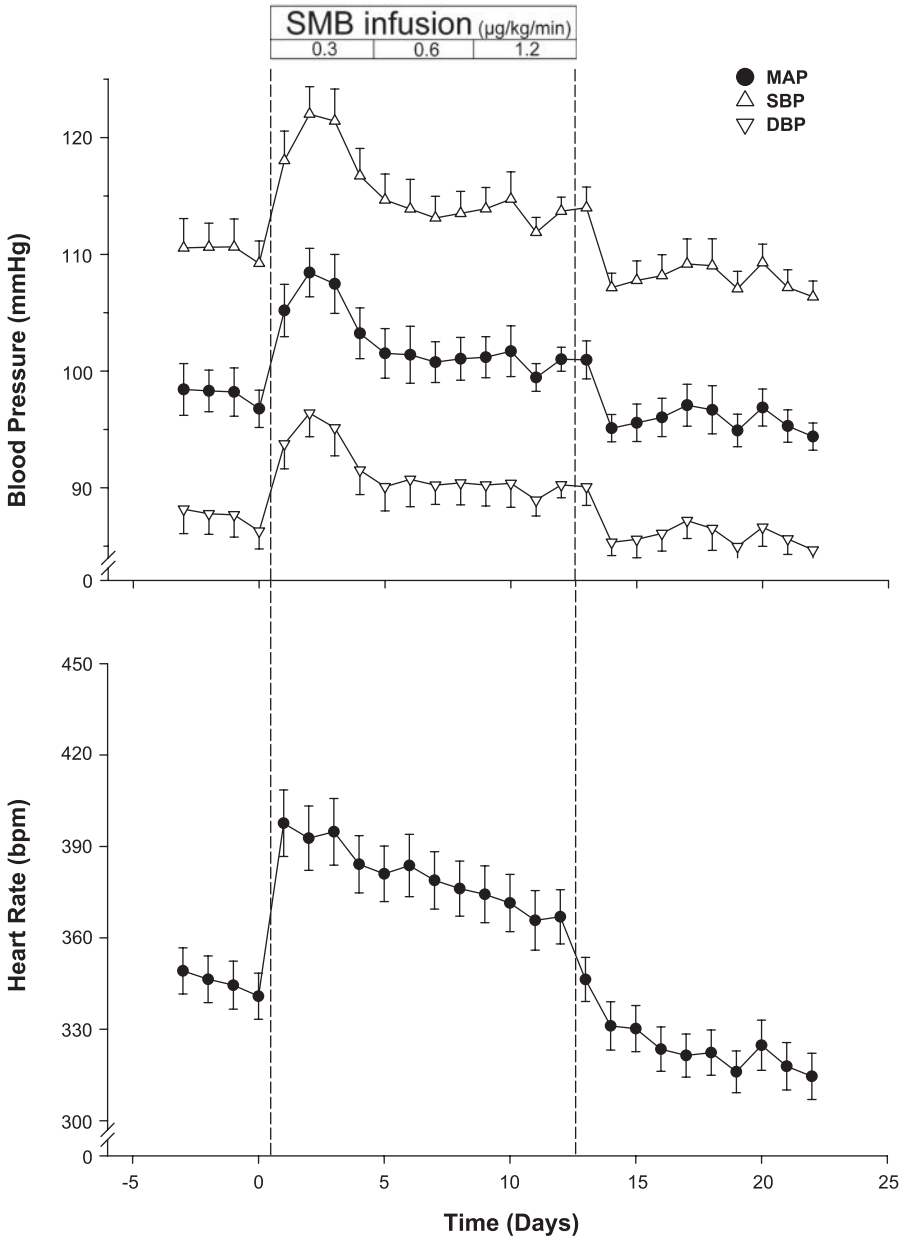

Fig. 1. Effects of a 12-day scopolamine methyl bromide (SMB) infusion on mean arterial pressure (MAP), systolic blood pressure (SBP), and diastolic blood pressure (DBP), and on heart rate (bpm, beats/min).

slightly on *day 2* of SMB infusion but was back to normal thereafter. This explains the transient decrease in sodium and potassium intakes during *day 2*. In these three rats the non-ingested sodium of *day 2* was compensated on the next day by supplementing food with an estimated equivalent of sodium chloride. Sodium and potassium balances remained stable over the following days.

DISCUSSION

Whereas numerous studies have reported the cardiovascular effects of acute parasympathetic inhibition, we are not aware of any published study of the effects of more sustained parasympathetic blockade on hemodynamic function. The main finding of our study is that chronic peripheral muscarinic blockade leads to significant hemodynamic alterations, including sustained elevations in BP, HR, and BPV.

The effect on BP was most pronounced during the first 3 days of muscarinic inhibition, with a peak increase in SBP, MAP, and DBP of more than 10% during *day 2* of SMB. Although BP tended to return toward control level over the following few days, BP remained significantly elevated during the last 4 days of SMB infusion as shown in Table 1, a conclusion also supported by the off transient in BP when the infusion was halted. The chronic SMB-induced effect on MAP amounted to a 3–6% increase, depending whether its effects are compared with the initial control period (3%) or the final recovery period (6%).

The partial recovery of blood pressure during SMB infusion occurred despite evidence of the continued effectiveness of muscarinic blockade by SMB. HR, which initially increased by as much as 15% above control value, remained elevated by 11% on the last day of SMB infusion (based on the size of the

Table 1. *Changes in hemodynamic values when compared with control levels*

Variable	*Period 1*	*Period 2*	*Period 3*	Recovery
SBP, mmHg	9.3±0.5†	3.5±0.9*	3.3±1.1*	−2.8±1.6
MAP, mmHg	8.2±0.4†	3.3±0.7†	2.9±0.7*	−2.6±1.1*
DBP, mmHg	6.7±0.5†	2.9±0.7*	2.5±0.5*	−2±0.9
HR, beats/min	47±5†	35±4†	24±4†	−27±6†
PP, mmHg	2.6±0.7*	0.7±0.8	0.8±1	−0.8±1.5
Max dP_a/dt, mmHg/s	579±184*	185±163	66±264	−138±260
Rise time, ms	−1.7±0.2*	−1.3±0.4*	−1.0±0.4	0.2±1

Values are means ± SE ($n = 7$ rats) computed as changes from control levels [last 4 days before starting scopolamine methyl bromide (SMB) infusion]. SBP, systolic blood pressure; MAP, mean arterial pressure; DBP, diastolic blood pressure; HR, heart rate; PP, pulse pressure; Max dP_a/dt, maximum rate of change of arterial pressure. *Periods 1*, *2*, and *3* correspond to *days 1–4*, *5–8,* and *9–12*, respectively, of SMB infusion. Recovery corresponds to *days 7-10* after cessation of SMB infusion. *$P < 0.05$ and †$P < 0.01$ vs. control period (Dunnett's).

off transient, *day 2* of recovery). The attenuation of the "short-term" HR variability was even more dramatic, responding promptly at the onset and end of SMB infusion and not wavering throughout the 12-day infusion period. Combined with the observation that doubling the SMB dosage on *days 5–8* (*period 2*) and again on *days 9–12* (*period 3*) failed to cause further alterations in HR, "short-term" HR variability, or any other measured parameter, our data suggest that the SMB infusion provided an effective and sustained blockade of cardiac muscarinic receptors.

The values of BP and HR during *days 7–10* of the recovery period (−2.6 ± 1.1 mmHg and −27 ± 6 beats/min below control values for MAP and HR, respectively) are consistent with a moderate spontaneous decrease in hemodynamic values often observed in chronic experiments after a surgical procedure. Indeed, in a group of vehicle-infused rats instrumented in a similar way, there was an average decrease in MAP and HR of 3% and 8%, respectively, from *days 9–12* after surgery to *days 25–28*. This slow decrease in vehicle-infused animals, possibly related to the gradual disappearance of expected postsurgery inflammatory reactions, strengthens our findings in SMB-infused rats that the significant increases in MAP and HR associated with long-term SMB infusion are not due to a slow positive baseline drift but represent a real phenomenon. Indeed, after the negative drift occurring in time-control animals was corrected, the estimated chronic effects of SMB amounted to a 6% increase in MAP and a 13% increase in HR (*period 3*).

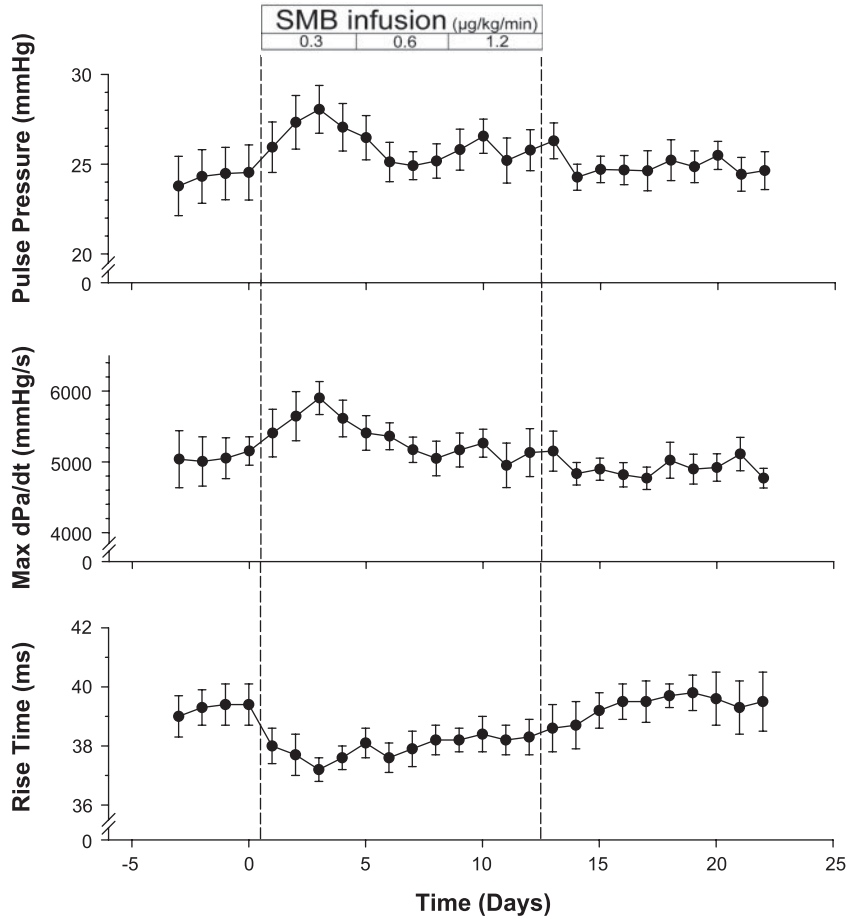

Fig. 2. Effects of a 12-day SMB infusion on pulse pressure, maximum arterial pressure change rate (dP_a/dt) and rise time.

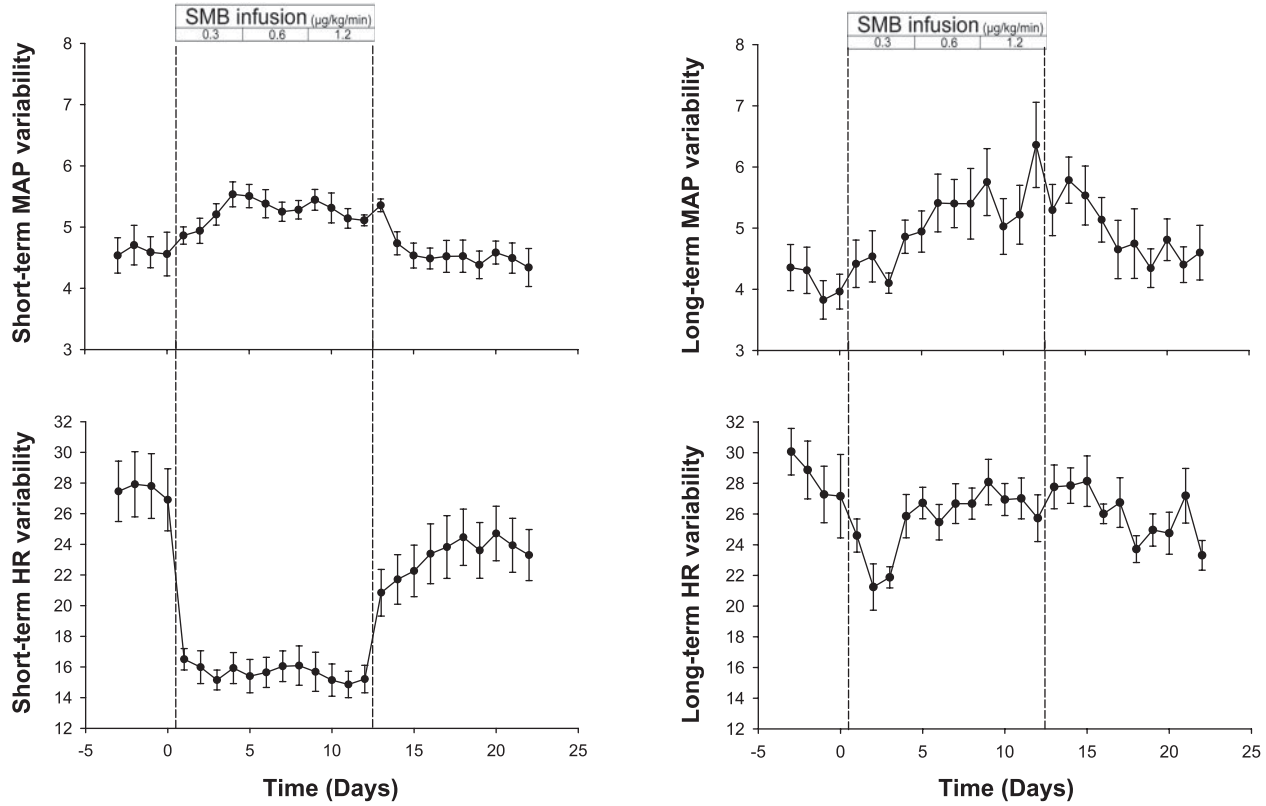

Fig. 3. Effects of a 12-day SMB infusion on short-term and long-term MAP and heart rate (HR) variabilities. The time course of short-term and long-term SBP variabilities was very similar to the time course of MAP variabilities and is therefore not shown.

Although our experimental approach (recording throughout the day, with animals left undisturbed as much as possible) did not allow a more precise determination of the mechanisms underlying the SMB-induced increase in BP, at least three factors could be involved: *1*) an increase in cardiac output (CO) due to a primary increase in HR and/or stroke volume; *2*) an increase in peripheral vascular resistance; and *3*) an altered ability of the kidney to excrete salt and water.

For the first factor, while an increase in HR is expected initially to increase CO, stroke volume tends to fall with time and changes in HR per se may not lead to sustained increases in CO (8). However, a combination of increased HR and contractility could potentially cause a larger increase in CO. Tachycardia alone is expected to decrease PP and maximum dP_a/dt as shown in heart-pacing experiments (26). In our experiments, the observed increases (and the lack of decreases) in PP and maximum dP_a/dt and the decrease in rise time are consistent with an increase in cardiac contractility during the SMB infusion, although we cannot exclude an effect of SMB to decrease arterial distensibility.

For second factor, muscarinic blockade could promote vasoconstriction. There is, however, little vascular parasympathetic innervation to be blocked except for sparse specialized circulations (33), but there is evidence for a local release of acetylcholine (ACh) by endothelial cells themselves. The presence of choline acetyltransferase, the enzyme responsible for the synthesis of ACh, has been shown in cerebral microvascular endothelial cells of rats (20) and pigs (11), as well as in bovine carotid artery endothelial cells (13). Milner et al. (16) could demonstrate a local release of ACh in isolated human umbilical vein endothelial cells, which is triggered by shear stress. Release of ACh by cultured endothelial cells prepared

Table 2. *Short-term (within 30 min), long-term (between 30 min), and overall (22 h) SD of SBP and MAP*

Variable	Control	*Period 1*	*Period 2*	*Period 3*	Recovery
SBP					
Short-term SD, mmHg	4.9±0.3	5.6±0.2*	5.9±0.2†	5.7±0.2†	4.8±0.2
Long-term SD, mmHg	4.5±0.3	5.0±0.3	5.9±0.4†	6.3±0.6†	4.9±0.4
Overall SD, mmHg	6.6±0.3	7.5±0.2*	8.3±0.4†	8.5±0.5†	6.9±0.4
MAP					
Short-term SD, mmHg	4.6±0.3	5.1±0.2*	5.4±0.2†	5.3±0.1†	4.5±0.2
Long-term SD, mmHg	4.1±0.3	4.5±0.2	5.3±0.4†	5.6±0.5†	4.5±0.3
Overall SD, mmHg	6.1±0.3	6.8±0.2*	7.5±0.3†	7.7±0.4†	6.3±0.3

Values are means ± SE ($n = 7$ rats). SD, standard deviation. Control represents the last 4 days before starting SMB infusion. *Periods 1, 2,* and *3* correspond to *days 1–4*, *5–8*, and *9–12*, respectively, of SMB infusion. Recovery corresponds to *days 7–10* after cessation of SMB infusion. *$P < 0.05$ and †$P < 0.01$ vs. control period (Dunnett's).

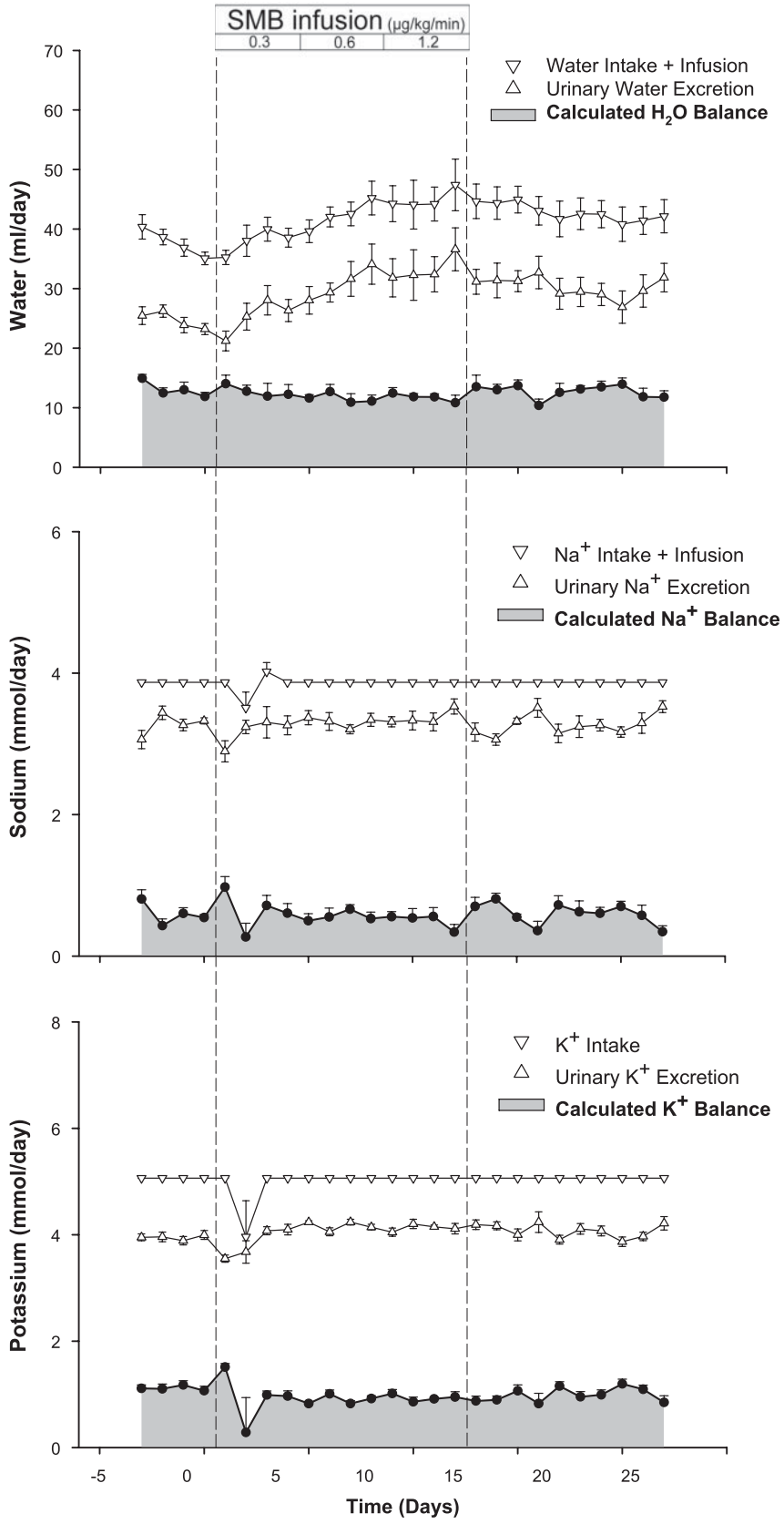

Fig. 4. Effects of a 12-day SMB infusion on the daily balance of water, sodium, and potassium. Water intake and sodium intake include the amount of water and sodium, respectively, contained in the intravenous infusion. Balance was computed as intake minus urinary excretion.

from bovine carotid artery was verified by radioimmunoassay (13). Taken together, these results show that endothelial cells can synthesize and release ACh, which may act as an autacoid and reinforce stretch-induced nitric oxide (NO) release. Inhibition of endothelial muscarinic receptors by SMB may thus impair stretch-induced NO release. Interestingly, the hypertensive effect of NO synthase inhibition is potentiated by acute cholinergic blockade (14), suggesting an interaction between cholinergic and nitrergic vasodilatation.

For the third factor, the kidneys may also be involved in the SMB increase in BP. Normally, a rapid increase in BP by vasoconstrictors would be expected to result in a pressure-induced natriuresis (10). Yet, no initial natriuresis was observed in our experiments despite a rise in BP of more than 10 mmHg, suggesting sodium-retaining forces that could offset pressure-induced natriuresis. ACh has a natriuretic effect on the kidney, which is inhibited by atropine (34). Although there is no clear parasympathetic innervation of the kidneys (5), the antagonism of endothelial mechanisms, as described above, could possibly promote renal vasoconstriction and sodium retention.

Finally, ACh is known to inhibit the release of norepinephrine from sympathetic nerve terminals via stimulation of prejunctional muscarinic receptors (36). Thus an additional possibility is that blockade of these receptors by SMB could effectively increased sympathetic tone at the level of the heart and vasculature.

Although a chronic increase in 24 h BP of 3–6% is physiologically relatively modest, human clinical trials indicate that sustained similar increases in BP would have substantial effects on cardiovascular risk. Furthermore, it is possible that the tonic influence of the parasympathetic nervous system on BP may play a larger role in humans or in other circumstances. Indeed, rats have a modest parasympathetic tone compared with larger animals such as dogs and humans, perhaps in part because they are usually studied at laboratory temperatures (~20–22°C), which are well below the range of ambient temperatures that requires minimal thermoregulatory effort to maintain body temperature (thermoneutrality zone). The thermal neutral zone of healthy rats of common strains in experimental setups similar to ours (single cage housing, no bedding) is around 30°C (23). Warming ambient temperature into the zone of thermoneutrality has been shown to reduce BP and HR and to increase HR variability in both rats and mice (28). The parasympathetic system may thus play a potential larger role in BP regulation in humans than in rats and mice maintained at standard laboratory temperatures.

Chronic muscarinic blockade also led to significant increases in short-term (within 30 min SD), long-term (between 30 min SD), and overall (22 h SD) BPV. Vagal dysfunction impairs the baroreflex control of HR, which in turn could lead to greater oscillations in BP and hence to an increased short-term BPV (7). Indeed, muscarinic blockade in our study led to a marked and sustained decrease in "short-term" HR variability and an increase in short-term BPV. The slow increase in long-term BPV observed during SMB infusion is intriguing, but the underlying mechanism remains unclear.

Increases in BPV could contribute to hypertensive target organ damage (TOD). Indeed, several clinical investigations have found TOD to be more advanced in patients with increased BPV (19, 21). These findings have been reinforced by a prospective investigation (9) in which patients that had taken part in one of the original studies (19) were followed up ~7 years later. The role of systolic BPV in promoting TOD has been confirmed in large clinical studies, such as the Pressioni Arteriose Monitorate E Loro Associazioni (PAMELA) study (25) and the Syst-Eur trial (22).

Perspectives. Parasympathetic dysfunction is not a rare event. In addition to hypertension itself, reductions in cardiac parasympathetic tone or reactivity have been described in obesity (1, 31), diabetes mellitus (3), chronic renal failure (2), physical inactivity (24), and ageing (27). Other factors may promote parasympathetic dysfunction via direct muscarinic receptor inhibition. Autoantibodies against muscarinic receptor, of which its occurrence increases with age, have been found in healthy subjects (15). Moreover, some major drugs, such as neuroleptics, antiarrhythmics, and antidepressants may have anticholinergic effects.

Whereas a contribution of parasympathetic dysfunction to cardiovascular morbidity has been suggested by many authors, the hemodynamic effects of chronic muscarinic blockade have not been reported. Our study shows that chronic muscarinic receptor inhibition favors a small increase in BP and leads to a significant increase in both short-term and long-term BPV. Our data suggest that chronic peripheral muscarinic blockade could contribute to the development or aggravation of hypertension and TOD.

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