Effects of β-adrenergic receptor agonists on drinking and arterial blood pressure in young and old rats

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Thunhorst RL, Grobe CL, Beltz TG, Johnson AK. Effects of β-adrenergic receptor agonists on drinking and arterial blood pressure in young and old rats. Am J Physiol Regul Integr Comp Physiol 300: R1001–R1008, 2011. First published February 9, 2011; doi:10.1152/ajpregu.00737.2010.—These experiments examined water-drinking and arterial blood pressure responses to β-adrenergic receptor activation in young (4 mo), “middle-aged” adult (12 mo), and old (29 mo) male rats of the Brown-Norway strain. We used isoproterenol to simultaneously activate β1- and β2-adrenergic receptors, salbutamol to selectively activate β2-adrenergic receptors, and the combination of isoproterenol and the β2-adrenergic receptor antagonist ICI 118,551 to stimulate only β2-adrenergic receptors.

Several studies have used isoproterenol to examine age-related declines in drinking by rats (24, 25, 34, 35). However, the relative contributions of the β1- and β2-adrenergic receptor subtypes to age-related drinking have not been examined. Old rats have greatly impaired baroreflex mechanisms (2, 9, 18, 32, 38) and respond to β-adrenergic receptor activation with diminished tachycardia (1, 6, 35; but see Ref. 9) and attenuated renin secretion (4), which may affect drinking differently in old and young rats. In this study, we assessed drinking, arterial pressure, and HR responses after subcutaneous injection of isoproterenol in young (4 mo), “middle-aged” adult (12 mo), and old (29 mo) rats. Contributions of the β1-adrenergic receptor subtype to these responses were examined by administering isoproterenol in combination with the selective β2-adrenergic receptor antagonist, ICI 118,551. The effects of β2-adrenergic receptor activation on these responses were determined by administering the selective β2-adrenergic receptor agonist salbutamol. We also measured plasma renin levels after treatment with isoproterenol and salbutamol.

METHODS

Animals. Male Brown Norway rats aged 3 mo (young), 11 mo (“middle-aged” adult), and 28 mo (old) were obtained from Harlan (Indianapolis, IN) through services provided by the National Institute on Aging (NIA). All rats were housed singly in hanging stainless-steel cages in a temperature-controlled room (23°C) on a 12:12-h light-dark cycle. They received ad libitum access to standard Teklad rodent diet and tap water. Animals were allowed 1 mo for adaptation and were tested at 4, 12, and 29 mo of age, respectively. All procedures were approved by the University of Iowa Institutional Animal Care and Use Committee.

Drugs. The mixed β1,β2-adrenergic receptor agonist isoproterenol (Isuprel, Hospira, Lake Forest, IL) was diluted 3:1 with isotonic saline and administered subcutaneously at a dose of 15 μg/kg body wt per injection. The β2-adrenergic receptor agonist salbutamol (Sigma-Aldrich, St. Louis, MO) was dissolved in isotonic saline and administered subcutaneously at a dose of 150 μg/kg body wt per injection. The β2-adrenergic receptor antagonist ICI 118,551 (ICI; Sigma-Aldrich) was dissolved in isotonic saline and administered subcutaneously at a dose of 1 mg/kg body wt.

Catheter surgery. Rats received femoral arterial catheters under isoflurane (Phoenix Pharmaceutical, St. Joseph MO) anesthetic for measurement of arterial pressure. Catheters were made from polyethylene tubing (PE-50) ~20 cm in length that was heat welded to a

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shorter piece of PE-10. The PE-10 was inserted into the vessel and advanced 3.5 cm. The catheters were tunneled under the skin, secured between the scapulae, and exteriorized at the base of the neck. When not in use, the catheters were filled with heparinized saline (200 U/ml) and plugged with 23-gauge obturators.

Blood pressure recording. The analog signal from a Cobe pressure transducer was processed with a CED 1401 laboratory interface (Cambridge Electronics Design, Cambridge, England) using Spike 5 software (Cambridge Electronics Design) on a Dell Optiplex GX620 Pentium D computer. The data were analyzed by a script file that used the digitized pulsatile pressure to calculate mean arterial pressure (MAP), HR, and systolic and diastolic pressures. The data were averaged over 5-min bins. Resting MAP was determined using the last 15 min of the baseline period before injection of the drugs.

Drug injection protocol. The drinking and cardiovascular tests included two injections of isoproterenol or of salbutamol spaced 45 min apart rather than single injections of the drugs. In preliminary work, we observed that a dose of isoproterenol (i.e., 30 μg/kg body wt) that is typical of doses employed in drinking tests sometimes produced adverse reactions (e.g., “wet dog” shakes) in the oldest animals. Reducing this dose by half eliminated obvious adverse reactions in the older animals—indeed, they now handled repeated testing well—but yielded smaller intakes by animals of all ages. Therefore, since arterial pressure and HR largely recovered by 45 min postinjection of isoproterenol (e.g., Fig. 2; Refs. 23 and 35), we included a second injection at that time to prolong the drug effects and produce more drinking. For consistency, all drug treatment conditions included two subcutaneous injections of isoproterenol or salbutamol that were spaced 45 min apart. All experimental testing was conducted between 9:00 a.m. and 3:00 p.m.

Drinking tests. Rats were tested for drinking responses in their home cages. One cohort of 4-, 12-, and 29-mo-old rats received two subcutaneous injections, spaced 45 min apart, of isoproterenol (15 μg/kg body wt per injection). A second cohort received two subcutaneous injections, spaced 45 min apart, of salbutamol (150 μg/kg body wt per injection). A third cohort received a subcutaneous injection of ICI 118,551 (1 mg/kg body wt), followed 15 min later by two subcutaneous injections of isoproterenol, spaced 45 min apart. Chemical bourets with drinking spouts were attached to the front of the cages immediately after the first injection of isoproterenol or salbutamol, and water intakes were recorded every 15 min for 90 min. Food was not available during testing.

Blood pressure tests. Rats received femoral artery catheters 2–4 days after the conclusion of the drinking tests, and blood pressure testing began 2–3 days later. Blood pressure tests were performed in a separate room. The test cages were wooden with aluminum-lined interiors (24 × 29 cm) that extended 31 cm above suspended, stainless-steel metabolism cages. On the morning of testing, rats were brought to the experimental room, weighed, and placed in the test cages. Lengths of PE-50 tubing were used to connect the arterial lines to pressure transducers, and arterial blood pressure was recorded continuously. Water was not available for drinking. Rats were allowed 60–75 min to acclimate and establish resting measures. Next, they received either two subcutaneous injections, spaced 45 min apart, of isoproterenol or salbutamol, or a subcutaneous injection of ICI 118,551 followed 15 min later by two subcutaneous injections of isoproterenol spaced 45 min apart. The rats received the same drug treatment they had received for the drinking test.

Blood collection. Approximately 1 wk after completion of blood pressure testing, trunk blood was collected from some of the rats for determination of hematocrit, plasma renin activity (PRA), and plasma aldosterone levels. For these measures, rats in the isoproterenol cohort were administered isoproterenol and those in the isoproterenol + ICI cohort were administered salbutamol. Testing occurred in the home cage. Water was not available for drinking. Rats received a single subcutaneous injection of isoproterenol (15 μg/kg body wt) or salbutamol (150 μg/kg body wt) in a manner identical to the first injection of drug in the behavioral and cardiovascular tests and were decapitated 30 min later for collection of trunk blood. Trunk blood was collected in chilled tubes containing EDTA (final concentration 2 mg/ml blood) for centrifugation at 3,000 g for 10 min at 4°C. The plasma was stored at −80°C for measurement of hormones at a later date. A small amount of trunk blood was collected by microhematocrit tubes for centrifugation and measurement of hematocrit in triplicate.

Plasma aldosterone and plasma renin activity measurements. Levels of aldosterone were measured using Diagnostics Products solid-phase 125I radioimmunoassay kits (Diagnostics Products, Los Angeles, CA). The sensitivity for aldosterone is ~16 ng/ml. PRA was determined by measuring the formation of ANG I using a commercially available radioimmunoassay kit (Perkin Elmer, Waltham, MA). Levels of ANG I accumulated under the conditions of the assay by allowing the endogenous substrate to react in the presence of reagents that inhibit plasma converting enzymes and angiotensinas. The sensitivity for PRA is <1 ng·ml−1·min−1. The intra-assay and inter-assay coefficients of variation are 8 and 10%, respectively.

Statistical analysis. Data were analyzed by one-way, univariate, or repeated-measures ANOVA. Analysis of the drinking data was conducted on the raw, noncumulated measures at each time point (13). Planned comparisons were made with Fisher’s least-significant difference tests when the global F ratio was significant or with Bonferroni tests when the global F ratio was not significant. Values were considered significant at the P < 0.05 level. The results are expressed as means ± SE.

RESULTS

Body weights at the start of the drinking tests differed significantly by age (the means ± SE were 257 ± 6, 387 ± 5, and 462 ± 9 g at 4 mo, 12 mo, and 29 mo, respectively; F2, 72 = 176.38, P < 0.001) but not by drug treatment. Therefore, water intake was analyzed as rate of intake, i.e., ml/15 min of testing, both as absolute and as body weight-adjusted (i.e., ml/100 g) measures. We used a mixed-model ANOVA with time as the within-subjects repeated measure and age and drug treatment as between-subjects variables. For absolute intakes, there was a significant main effect of treatment (F2, 72 = 54.65, P < 0.001), and all interactions were significant, including a significant 3-way interaction of time × age × treatment (F20, 360 = 1.61, P < 0.05). Drinking in response to isoproterenol and salbutamol was nearly identical and was virtually absent after combined treatment with isoproterenol + ICI. Rats drank more in response to both isoprote- renol and salbutamol than to isoproterenol + ICI in most measurement periods. Young rats drank more than both other groups after isoproterenol and salbutamol, due mostly to the significantly increased drinking by young rats in the second 15-min period following each injection of the drugs, which was not observed in middle-aged and old rats. For body weight-adjusted measures, all main effects and interactions were significant. Body weight-adjusted water intakes are presented both as cumulative and noncumulative intakes in Fig. 1. As in the prior analysis, drinking by all groups was essentially absent after combined treatment with isoproterenol + ICI, and nearly equivalent in response to isoproterenol and salbutamol (treatment main effect, F2, 78 = 30.60, P < 0.001). Overall, young rats drank more than middle-aged and old rats (age main effect, F2, 78 = 9.50, P < 0.001), and this was due to the significantly increased drinking in response to isoproterenol and salbutamol by young rats compared with both other groups (age × treatment interaction, F4, 72 = 8.95, P < 0.001). As in the prior analysis, the increased rate of drinking by young rats occurred.
primarily in the second 15-min period after each injection of isoproterenol and within 30 min of each injection of salbutamol (time × age × treatment interaction, $F_{20, 360} = 1.90, P < 0.05$).

The MAP and HR data were analyzed by ANOVA using time as the within-subjects repeated-measure where appropriate and age and drug treatment as between-subjects factors. The data for MAP and HR are presented in Fig. 2 and Table 1. A main effect of age ($F_{2, 47} = 6.20, P < 0.01$) revealed that resting MAP was significantly higher for old rats compared with the other groups. There were no other effects for resting MAP. Resting HR was significantly higher during salbutamol testing compared with the other drug tests (treatment main effect, $F_{2, 47} = 23.72, P < 0.001$). Indeed, MAP averaged across the groups was essentially unchanged when ICI compound was administered before isoproterenol. Reductions in arterial pressure were also greater with increasing age (age main effect, $F_{2, 47} = 10.46, P < 0.001$). Planned contrasts revealed age differences in the changes in arterial pressure from baseline, averaged over the entire 90 min of testing, following administration of isoproterenol and isoproterenol + ICI (i.e., reductions in MAP after isoproterenol were significantly greater for old rats compared with young rats) but not following administration of salbutamol. Heart rate changed significantly less in response to all treatments in the old rats compared with both other groups (age main effect, $F_{2, 47} = 18.71, P < 0.001$). In addition, HR changed significantly less in response to salbutamol than in response to the other treatments (treatment main effect, $F_{2, 47} = 13.37, P < 0.001$).

Plasma levels of hematocrit, renin activity, and aldosterone were analyzed by univariate ANOVA with age and treatment as fixed factors (Fig. 3). Hematocrit was reduced in old animals compared with young and middle-aged rats and did not differ between young and middle-aged rats (age main effect, $F_{2, 43} = 3.30, P < 0.05$). There were no significant treatment effects on PRA. For plasma aldosterone levels, there was no significant main effect of age. However, planned contrasts revealed that old rats had significantly reduced plasma levels...
of aldosterone in response to isoproterenol compared with either young or middle-aged rats. A main effect of treatment showed that plasma levels of aldosterone were significantly greater after salbutamol than after isoproterenol ($F_{1,43} = 4.08$, $P < 0.05$), but this result was due to the significantly reduced plasma levels of aldosterone in the old rats after isoproterenol.

**DISCUSSION**

The major results of the present work were these: 1) Young (4 mo) rats drank more water than did “middle-aged” adult (12 mo) and old (29 mo) rats after treatment with the mixed $\beta_1, \beta_2$-adrenergic receptor agonist, isoproterenol, the selective $\beta_2$-adrenergic receptor agonist, salbutamol, and the combination of isoproterenol and the $\beta_2$-adrenergic receptor antagonist, ICI 118, 551 (ICI) in young (4 mo), middle-aged (12 mo), and old (29 mo) rats. Values are expressed as means ± SE. There were significant effects of age on changes in MAP after isoproterenol and isoproterenol + ICI. Old rats had significantly smaller changes in HR after all treatments compared with the other groups. Group n's for young, middle-aged, and old rats were isoproterenol = 8, 6, 6; salbutamol = 6, 5, 5, and isoproterenol + ICI = 7, 5, 8, respectively.

**Fig. 2.** Change in mean arterial pressure (Δ MAP) and heart rate (Δ HR) after injections of the mixed $\beta_1, \beta_2$-adrenergic receptor agonist, isoproterenol, the selective $\beta_2$-adrenergic receptor agonist, salbutamol, and the combination of isoproterenol and the $\beta_2$-adrenergic receptor antagonist, ICI 118, 551 (ICI) in young (4 mo), middle-aged (12 mo), and old (29 mo) rats. Values are expressed as means ± SE. There were significant effects of age on changes in MAP after isoproterenol and isoproterenol + ICI. Old rats had significantly smaller changes in HR after all treatments compared with the other groups. Group n's for young, middle-aged, and old rats were isoproterenol = 8, 6, 6; salbutamol = 6, 5, 5, and isoproterenol + ICI = 7, 5, 8, respectively.
fore, reduced renin secretion may not entirely explain the age-related differences in drinking reported here.

The core finding of the present work was age-related declines in drinking in response to combined β1,β2-adrenergic receptor activation with isoproterenol and selective β2-adrenergic receptor activation with salbutamol. The diminished drinking responses to both drugs by middle-aged and old rats compared with young rats were evident whether analyzing absolute or body weight-adjusted measures. Increased drinking by young rats occurred as pronounced surges in the rates of drinking about 30 min after each injection of the drugs and coincided with elevated levels of PRA in young rats compared with the older animals. Middle-aged and old rats did not have pronounced surges in drinking after the drugs and had greatly reduced renin levels compared with young rats. A likely explanation for drinking after isoproterenol and salbutamol is that β2-adrenoceptor activation by the drugs causes vasodilation and reductions in arterial pressure that stimulate drinking by releasing renal renin (17, 21, 23, 26, 31). Signals arising from arterial baroreceptors in response to hypotension are postulated to stimulate drinking after isoproterenol (16, 31). In the present study, isoproterenol-induced drinking appears to be mediated primarily by β2-adrenergic receptors. First, antagonism of β2-adrenergic receptors with ICI abolished drinking in response to isoproterenol across the age groups. The fact that arterial pressure, averaged across the groups, did not change after isoproterenol + ICI is presumptive evidence that β2-adrenergic receptors were functionally blocked by the ICI compound (21). In addition, the increases in HR after isoproterenol + ICI, which are nearly indistinguishable from the increases in HR produced by isoproterenol administered alone, indicate that myocardial β1-adrenergic receptors were not blocked by ICI (21). Therefore, the lack of drinking after treatment with isoproterenol + ICI, despite evidence of β1-adrenergic receptor activation, suggests that stimulation of β1-adrenergic receptors is not necessary for isoproterenol-induced drinking, and this conclusion holds across age. Second, selective β2-adrenergic receptor activation with salbutamol produced as much drinking at all ages as did isoproterenol. Falk and Tang (10) were the first to show that salbutamol caused water drinking by rats. Subsequently, Katovich and Fregly (20) used the copious drinking that they observed with salbutamol to suggest that activation of β2-adrenergic receptors could explain drinking in response to isoproterenol. Our present results confirm the dispogenic nature of salbutamol in rats and extend these findings by showing that drinking responses to both isoproterenol and salbutamol decline with age. Together, these findings suggest that isoproterenol-induced drinking depends largely on activation of β2-adrenergic receptors. However, we cannot discount an important role for β1-adrenergic receptors, as we did not specifically test their contribution to isoproterenol-induced drinking using β1-adrenergic receptor antagonists. Notably, in experiments by Kirby et al. (21), selective blockade of either β1- (i.e., with atenolol) or β2- (i.e., with ICI) adrenergic receptors greatly reduced, but did not eliminate, renin secretion and drinking in response to isoproterenol. In addition, both the amounts of water intake and renin secretion stimulated by isoproterenol alone were greater than the sum of those stimulated by activating either receptor subtype separately (i.e., when isoproterenol was administered with β1- or β2-adrenergic receptor antagonists). Therefore, Kirby et al. (21) argued that renin secretion and drinking after

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<th>Change in MAP, mmHg</th>
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<tr>
<td></td>
<td>Isop</td>
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<tr>
<td>4 mo</td>
<td>103 ± 2</td>
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<td>12 mo</td>
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<tr>
<td>29 mo</td>
<td>113 ± 4</td>
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<td>Total</td>
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Values are expressed as means ± SE. Resting measures are averaged over the last 15 min before injection of the mixed β1,β2-adrenergic receptor agonist, isoproterenol (Isop), the selective β2-adrenergic receptor agonist, salbutamol (Salbut), and the combination of isoproterenol and the β2-adrenergic receptor antagonist, ICI 118,551 (ICI). Change measures are the average differences from resting values for the entire 90-min period after drug injection. MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute. *Significantly different from young rats, P < 0.05. †Significantly different from both other groups, P < 0.05.
isoproterenol result from a synergy of the direct effects of activating juxtaglomerular \( \beta_1 \)-adrenergic receptor agonists and the indirect effects of activating vascular \( \beta_2 \)-adrenergic receptors and ensuing hypotension. In support of this idea, they noted other studies showing that hypotension facilitates renin release during renal nerve stimulation (36) and increases drinking in response to \( \text{ANG II} \) (37). Thus, a key component of isoproterenol-induced renin secretion and drinking may be sensitivity to the modulatory effects of arterial pressure on these responses.

Unlike young animals, the drinking responses of older animals are remarkably unaffected by changes in arterial pressure (39). In previous work, we have shown that young animals drink more water in response to intravenous infusion of \( \text{ANG II} \) under conditions when MAP is reduced compared with when MAP is elevated (39). In contrast, we found that middle-aged and old rats drink similar amounts of water in response to intravenous infusion of \( \text{ANG II} \), regardless of level of MAP. In light of the mechanism of isoproterenol-induced drinking proposed by Kirby et al. (21), drinking—and renin secretion—by young animals in the present work may reflect facilitatory effects of hypotension on these responses, mediated by baroreflex mechanisms or other factors that change with arterial pressure. In contrast, older animals with impaired baroreflex mechanisms may be relatively insensitive to the influences of arterial pressure on these responses. Therefore, hypotension during isoproterenol or salbutamol might facilitate renin secretion and drinking more in young rats than in middle-aged or old rats.

The age-dependent levels of hypotension produced by isoproterenol appear to be the sum of the changes in MAP observed after activation of \( \beta_1 \)- (i.e., isoproterenol + ICI) and \( \beta_2 \)- (i.e., salbutamol) adrenergic receptors separately (Table 1). These age differences in isoproterenol-induced hypotension may reflect an interaction between the tachycardic responses and renin levels. Renal renin release is the rate-limiting step for generation of the powerful vasoconstrictor, \( \text{ANG II} \). The age-related reductions in renin secretion after isoproterenol and salbutamol suggest there was more circulating \( \text{ANG II} \) to maintain levels of arterial pressure in young rats, followed by middle-aged rats and, lastly, by old rats. The greater tachycardia observed after \( \beta_1 \) - or \( \beta_2 \)-adrenergic receptor activation in young and middle-aged animals also favors their ability to better maintain arterial pressure compared with old rats.

Therefore, an unexpected finding was the identical reductions in arterial pressure across the age groups following treatment with salbutamol. Vasodilatory drugs (e.g., nitroprusside, minoxidil) typically cause greater hypotension in older, compared with younger, rats, which is attributable to impaired baroreflex function and diminished renin secretion in older animals (9, 38). Despite comparable hypotension in all three groups, salbutamol clearly produced less tachycardia in old rats, and progressively reduced renin secretion in middle-aged and old rats compared with young rats. Therefore, the resulting physiological conditions favored the production of different levels of hypotension across age. We currently have no explanation for the finding of equivalent hypotension across the age groups in the face of the different levels of tachycardia and renin secretion.

On the basis of hematocrit levels, old rats had significantly greater relative plasma volume after injections of isoproterenol and salbutamol than young and middle-aged rats. When arterial pressure decreases, intravascular volume increases as Starling forces cause entry of fluid into the vascular space. Because arterial pressure following the drug treatments was generally lower in old rats than in young and middle-aged rats, it is likely that relatively more extracellular fluid entered the vasculature in old rats than in the other groups. In addition, old rats may be more susceptible to volume changes when arterial pressure is reduced because of increased venous compliance in old age (14).

Our findings that plasma renin levels were reduced by half in middle-aged rats compared with young rats and were virtually absent in old rats, after both isoproterenol and salbutamol, are consistent with several previous observations (3, 4, 5, 33). For example, acute administration of angiotensin-converting enzyme inhibitor increases PRA only one-third as much in old (18–20 mo) as in young (3–5 mo) Sprague-Dawley rats (5). Air jet stress increases PRA 10-fold above resting levels in young rats without appreciably increasing PRA in old rats (4).
Notably, the PRA response to air jet stress is abolished by β-adrenergic receptor blockade and thus appears to be β-adrenergic receptor-mediated (4). Old (20–24 mo) rats also have blunted PRA responses to prolonged dietary sodium restriction (19) or chronic treatment with angiotensin-converting enzyme inhibitor (27, 33; but see also Ref. 34). In contrast to the progressive age-related declines in renin secretion to both drugs, only old rats had attenuated aldosterone secretion after isoproterenol and both middle-aged and old rats retained their ability to secrete aldosterone after salbutamol. Increased PRA and, presumably, subsequent formation of ANG II in the circulation, after isoproterenol and salbutamol should stimulate aldosterone secretion. However, the levels of aldosterone in response to the drugs are not proportional to the renin levels, and, therefore, are unlikely to be explained entirely by levels of renin and ANG II in the present case. It has been shown that β-adrenergic agonists release aldosterone from adrenal zona glomerulosa cells in vitro (29, 30) and that this release is blocked by β₁- or β₂-adrenergic receptor antagonists (8, 28). Therefore, it is possible that the aldosterone levels in the present experiment resulted from direct effects of the drugs on β-adrenergic receptors in the adrenal glands, and that β₂-, but not β₁-, adrenergic stimulation of aldosterone secretion remains largely intact in older animals.

The HR responses to salbutamol are less, overall, than to isoproterenol. This likely reflects the different mechanisms of activation of HR by the two drugs. The relatively greater tachycardia observed after isoproterenol is likely due to direct pharmacological activation of myocardial β₁-adrenergic receptors, while the smaller tachycardic responses after salbutamol reflect increases from baroreflex mechanisms responding to the rather modest levels of hypotension obtained in the tests. Old rats had diminished levels of tachycardia after selective β₂-adrenergic receptor activation with salbutamol and also after combined treatment with isoproterenol + ICI. In the latter case, β₂-adrenergic receptors were presumably blocked by the ICI compound, so that isoproterenol-induced changes in HR were likely due to activation of unblocked myocardial β₁ receptors. Thus, old rats had a diminished HR response to either β₁- or β₂-adrenergic receptor activation in the present work.

Old rats have greatly impaired baroreflex-mediated HR responses (2, 9, 18, 32, 38). Simpkins et al. (35) found greatly diminished isoproterenol-induced tachycardia in old rats, but equivalent levels of tachycardia in middle-aged and young rats. Bunag and Teravainen (6) found attenuated tachycardia in old rats compared with young rats, despite equivalent depressor responses to isoproterenol. Therefore, they concluded that isoproterenol-induced tachycardia reflects direct myocardial β₁-adrenergic stimulation rather than reflex activation from hypotension. The attenuated tachycardia that we observed in old rats compared with young rats after isoproterenol was in the face of greater reductions in arterial pressure in the old rats. In addition, both the pattern and magnitude of the tachycardic response to isoproterenol were similar to those observed after treatment with isoproterenol + ICI (Fig. 3), which preferentially activated myocardial β₁ receptors. These results lend further support to the idea that changes in HR following administration of isoproterenol are mediated primarily by β₁-adrenergic receptors and are diminished in older animals. On the other hand, Docherty et al. (9) found that old animals had impaired baroreflex-mediated tachycardia in response to hypotension caused by nitroprusside but had intact myocardial β₁-adrenergic receptor-mediated tachycardia in response to isoproterenol. A major difference between these studies is that Docherty et al. administered isoproterenol to anesthetized and pithed rats, while we used conscious freely moving animals.

The present experiments did not include test conditions that assessed the effects of serial injections of vehicle. As noted, the rats in these studies showed no obvious adverse reactions to the injections of the drugs per se, or to repeated testing with the drugs. Nevertheless, it is possible that repeated stress from multiple injections, either within a test session or in a series of repeated tests, may be a factor that influenced the observed age-related differences in the present studies, and may be a factor to be considered in future work.

In summary, middle-aged and old rats drank significantly less water than did young rats after combined β₁,β₂-adrenergic receptor activation with isoproterenol and after selective β₂-adrenergic receptor activation with salbutamol. Reduced drinking by middle-aged and old rats was accompanied by equivalent, or greater, levels of hypotension, and by reduced, or absent, levels of plasma renin compared with levels of hypotension and plasma renin observed in young animals. However, reduced drinking by middle-aged and old rats cannot be explained simply as a function of diminished ability to secrete renin compared with young rats. Rather, reduced drinking by older animals may reflect both age-related impairments in renin secretion and relative insensitivity to the modulatory effects of arterial pressure on drinking.

**Perspectives and Significance**

The aging process is associated with diminished ability to sense and respond to behavioral and physiological stressors. However, it is becoming increasingly apparent that sensory and effector mechanisms, for example, those responsible for regulating body fluid and cardiovascular homeostasis, do not deteriorate with age at the same rates. The present work shows that old rats are much less capable than young rats, but just as capable as middle-aged rats, of drinking in response to potent β-adrenergic receptor activation despite having greatly impaired heart rate responses, and even greater blood pressure responses, to the same stimulus than do rats of the other ages. Furthermore, the abilities of hormonal systems to respond to β-adrenergic receptor activation decline across age with different rates. Lastly, the present work provides additional evidence of the utility of using selective β-adrenergic receptor agonists and antagonists to parse the relative contributions of the declines in direct and reflex activation of hormonal and behavioral systems to the overall reductions in drinking that accompany age.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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