Naunyn-Schmiedeberg's Arch Pharmacol 2005;371:99-106

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Effects of activation of central nervous histamine receptors in cardiovascular regulation; studies in H_1 and H_2 receptor gene knockout mice

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Abstract To elucidate the central roles of histamine receptors on cardiovascular regulatory system, systolic, mean, and diastolic blood pressures (BPs) and heart rate (HR) were examined in conscious H_1 receptor gene knockout (H_1KO) mice, H_2 receptor gene knockout (H_2KO) mice, H_1 and H_2 receptor gene double knockout (DKO) mice, and their respective control mice by the tail-cuff system. Histamine, histamine-trifluoromethyl-toluidine derivative (HTMT, an H_1 agonist), dimaprit (an H_2 agonist), and immepip (an H_3 agonist) were intrathecally administered to these KO mice and control mice.

Basal BPs and HR were not different among these three KO mice and their control or wild-type mice. Intrathecal administration of histamine significantly increased BPs and decreased HR in control mice. The increases in BPs were produced by histamine in H_1KO and H_2KO mice and by HTMT and dimaprit in C57BL mice. The pressor responses by HTMT and dimaprit in C57BL mice were greater than those by histamine in H_1KO and H_2KO mice, although the atropine-sensitive and same decreases in HR were induced by histamine in C57BL and H_1KO mice and by dimaprit in C57BL mice. The selective stimulation of H_3 receptors by immepip produced a consistent decrease in BPs in control mice.

These results suggest the sophisticated regulatory roles of the three histamine receptors and histaminergic neurons in the central cardiovascular system, that is, the pressor responses of histamine are mediated through the stimulation of both H_1 and H_2 receptors, whereas the decrease in heart rate is mainly due to the functions of H_2 receptor which activate the vagal output to the heart.

Keywords Blood pressure - Central cardiovascular system - Gene knockout mice - Heart rate - Histamine - H_1 receptors - H_2 receptors - H_3 receptors

Introduction

The presence of histaminergic neurons and at least three types of histamine receptors in the brain has been demonstrated (Watanabe et al. 1984; Panula et al. 1984; Schwartz J-C et al. 1991; Haas and Panula 2003), though the fourth histamine receptor is expressed in mainly the immune and inflammatory systems (Hofstra et al. 2003). The roles of histaminergic neurons and histamine receptors in integrative neuronal (e.g. arousal or homeostatic mechanisms) and behavioral (e.g. food intake, pain responses, learning or anxiety) functions have been recently reviewed (Brown et al. 2001; Watanabe and Yanai 2001; Haas and Panula 2003). The gene targeting techniques in mice have shown the elucidative abilities in various biological systems. We previously reported a behavioral characterization in mice lacking histamine H_1 receptors (Yanai et al. 1998; Mobarakeh et al. 2000; 2002).

Several studies indicate that the central administration of histamine results in a pressor response and a bradycardia in conscious animals (Klein and Gertner 1981; Poulakos and Gertner 1989; Brown et al. 2001). Both H_1 and H_2 receptors are involved in the pressor effects of histamine administered centrally (Poulakos and Gertner 1989; Brown et al. 2001). The decrease in heart rate depends exclusively on the activation of H_2 receptors (Poulakos and Gertner 1989; Brown et al. 2001). H_3 receptors modulate not only the histamine release as autoreceptors (Arrang et al. 1983; Arrang et al. 1987; Stark et al. 1996) but also the release of other neurotransmitters as heteroreceptors (Bealer SL 1993; Schlicker et al. 1994; Stark et al. 1996). However, the central cardiovascular effects of stimulation of H_3 receptors have not been elucidated yet until now, though there are various reports about their peripheral or other central roles

(Göthert et al. 1995; Malinowska et al. 1998; McLeod et al. 2003)

In this study, histamine and the receptor selective agonists, an H_1 agonist HTMT, an H_2 agonist dimaprit, and an H_3 agonist immepip, were intrathecally administered to examine the central cardiovascular effects of the stimulation of three types of histamine receptors. The BPs and HR in conscious mice were examined no invasively because anesthesia influences cardiovascular effects of histamine (Brown et al. 2001). Histamine receptors gene knockout mice were also used in this study because histamine antagonists may have some cardiovascular effects unrelated to blocking the histamine receptors (Gatti and Gertner 1984). The two kinds of experimental procedures, i.e. the use of selective agonists and the examination in gene knockout mice, will be very helpful to explore the central cardiovascular regulatory roles of the three histamine receptors.

Materials and methods

Animals. All experiments were performed in compliance with the relevant laws and institutional guidelines. Experimental protocols were approved be the Animal Care Committee of Tohoku University Graduate School of Medicine. Forty-six male mutant mice lacking histamine H_1 receptor (H_1KO mice) (21-32g at the beginning of the experiment), 37 male mutant mice lacking histamine H_2 receptor (H_2KO mice) (18-34g), 16 male mutant mice lacking both H_1 and H_2 receptors (DKO mice) (19-34g), 69 male C57BL mice (22-27g), and 40 male respective wild-type mice to H_1KO, H_2KO and DKO (21-30g), were used. The H_1KO, H_2KO, and DKO mice were backcrossed eight times to the C57BL mice. In this study, we used two different strains of the control mice, wild-type and C57BL mice. The male C57BL mice were purchased from Charles-River, Japan. The wild-type, H_1KO, H_2KO, and DKO mice were generated in our laboratories. All mice were housed in groups of 3-5 mice in glass metabolic cages at a controlled temperature (21 ± 1 ^{o}C) and humidity (55 ± 5 %) with a 12:12-h light-dark cycle. All animals had free access to standard pelleted chow and tap water. The genotypes of mice were analyzed by PCR of genomic DNA from tail biopsies with slight modifications in order to verify whether the histamine H_1 or H_2 or both H_1 and H_2 receptors were absent in mice as previously described (Inoue et al. 1996; Mobarakeh et al. 2000; Kobayashi et al. 2000).

Intrathecal Injection. Intrathecal injections (i.t.) were given by percutaneous lumber puncture through an intervertebral space at the level of the 5th or 6th vertebrate by the Hylden and Wilcox technique (Hylden and Wilcox 1980; Mobarakeh et al. 2000; 2002;

Murakami et al. 2001). Drugs were administered intrathecally in a volume of 5 μl using a 50-μl Hamilton microsyringe. A flick of the tail was used as an indication that the needle had penetrated dura. The injections were made in the rostral direction toward the brain. Histamine (3.2 nmol) in 5 μl artificial CSF was injected intravenously through the tail vein to check the possible effects of spillover to the periphery. The mice were not anesthetized during these procedures.

Cardiovascular recordings. The computerized, automated, tail-cuff system (BP-98A; Softron, Tokyo, Japan) were used for measurements of systolic blood pressure (SBP), mean blood pressure (MBP), diastolic blood pressure (DBP), and heart rate (HR). Each mouse was hold in a restrainer and pre-warmed at the temperature of 38.0 ^{o}C. Mice were not anesthetized during measurements (Murakami et al. 2000; 2003; Hagiwara et al. 2003). Six reliable recordings were used for determinations of BPs and HR.

Experimental procedure. BPs and HR were measured in mice before injection of drugs. All the baseline data obtained before injection of drugs were used to examine whether there can be any differences in BPs and HR between H_1KO, H_2KO, DKO, and their control mice. Histamine, an H_1 agonist HTMT, an H_2 agonist dimaprit, and an H_3 agonist immepip were intrathecally administered to C57BL mice, and histamine was intrathecally administered to H_1KO, H_2KO, DKO and their wild-type mice. BPs and HR were measured from 15 to 30 minutes after the injections. To examine the involvement of peripheral autonomic receptors in histamine agonists administered intrathecally, the effects of phenylephrine injected intraperitoneally (i.p.) were compared and atropine sulfate (1.4 nmol/g; 10 μl/g) or prazosin hydrochloride (0.72 nmol/g; 10 μl/g) was

injected i.p. 30 min before the i.t. of histamine or dimaprit. The i.p. injection of 0.9% NaCl saline did not change BPs and HR.

Solutions and drug administration. Drugs are obtained from the following sources: Histamine dihydrochloride, atropine sulfate, prazosin hydrochloride, and phenylephrine hydrochloride were obtained from Wako Pure Chemicals (Osaka, Japan). HTMT (6-[2-(4-imidazolyl)ethylamino]-N-(4-trifluoromethylphenyl) heptanecarboxamide), dimaprit, and immepip were purchased from Tocris Cookson (UK). For intrathecal injection, histamine, HTMT, dimaprit, and immepip were dissolved in sterile artificial cerebrospinal fluid (CSF) containing (mmol/l): NaCl 126.6, KCl 2.5, $MgCl_2$ 2.0, and $CaCl_2$ 1.3. Atropine sulfate, prazosin hydrochloride, and phenylephrine hydrochloride were dissolved in 0.9% NaCl. To increase the solubility of prazosin hydrochloride 1% of polyethylene glycol was added.

Statistical analysis. All results are expressed as mean ± S.E.M. with $P < 0.05$ considered as the level of significance. Changes in BPs and HR after the administration of histamine, HTMT, dimaprit, or immepip were compared by a paired Student's t-test. Differences in BPs and HR among C57BL, wild-type, H_1KO, H_2KO, and DKO mice in the absence or presence of agonists were analyzed by a two-way analysis of variance and compared by Scheffe test for statistical significance.

Results

Blood pressure and heart rate in C57BL, wild-type, H_1KO, H_2KO, and DKO mice.

There were no significant difference in SBP, MBP, DBP, and HR among H_1KO, H_2KO, DKO, their wild-type, and C57BL mice (Fig. 1). The means ± S.E.M. of baseline SBP of C57BL, wild-type, H_1KO, H_2KO, and DKO mice were 107.0 ± 0.8 (n=69), 107.5 ± 0.1 (n = 40), 108.4 ± 0.8 (n=46), 107.4 ± 1.1 (n=37), and 106.9 ± 1.8 mmHg (n=16), respectively. The means ± S.E.M. of baseline MBP of C57BL, wild-type, H_1KO, H_2KO, and DKO mice were 77.1 ± 0.5, 76.3 ± 0.5, 76.3 ± 0.5, 77.0 ± 0.9, and 77.5 ± 1.5 mmHg, respectively. The means ± S.E.M. of baseline DBP of C57BL, wild-type, H_1KO, H_2KO, and DKO mice were 62.1 ± 0.6, 60.8 ± 0.5, 60.3 ± 0.6, 62.0 ± 1.0, and 62.9 ± 1.7 mmHg, respectively. The means ± S.E.M of baseline HR of C57BL, wild-type, H_1KO, H_2KO, and DKO mice were 499.5 ± 4.4, 495.0 ± 3.9, 495.0 ± 5.9, 504.6 ± 6.6, and 499.9 ± 11.1 beats/min (bpm), respectively.

Cardiovascular effects of intrathecally-administered histamine.

When histamine was intrathecally injected to C57BL mice in a dose of 3.2 nmol, it produced increases in SBP, but a decrease in HR as shown in Fig. 2A and B. The pressor effects of histamine were maintained till 30 min after the injection. Intrathecal administration of CSF (5 μl) did not produce any significant changes in BPs or HR (SBP: from 106.9 ± 2.0 to 110.3 ± 2.0 mmHg, HR: from 490.2 ± 8.7 to 482.8 ± 12.2,

n=6). The intravenous injection of 3.2 nmol (around 0.1 nmol/g) histamine produced a slight decrease in SBP (from 105.2 ± 1.3 to 101.6 ± 1.0, n=6; $P<0.05$), but no change in HR (from 497.3 ± 9.4 to 488.3 ± 7.7, n=6) in C57BL mice. The dose-response relation of histamine was shown in Fig. 2C and D. A dose of 1.0 nmol of histamine evoked 16.5 ± 4.4, 11.3 ± 0.9, and 8.8 ± 1.3 mmHg increases in SBP, MBP, and DBP, respectively, and 31.1 ± 5.6 bpm decrease in HR (n=6). The dose of 3.2 nmol histamine produced 25.2 ± 5.0, 21.2 ± 2.0, 19.3 ± 2.6 mmHg rise in SBP, MBP, and DBP; 47.4 ± 5.0 decrease in HR (n=6). A dose of 10 nmol histamine, however, evoked 19.9 ± 3.9, 14.8 ± 1.8, and 12.2 ± 2.8 mmHg increase in SBP, MBP, and DBP; 35.7 ± 7.0 bpm decrease in HR (n=6). Thus, the peak effects of histamine were obtained at 3.2 nmol.

The summarized data of the cardiovascular responses to 3.2 nmol histamine (i.t.) in five mice groups are presented in Fig. 3. The effects on BPs and HR in wild-type mice were almost the same as those in C57BL mice. These data suggest that the cardiovascular effects caused by histamine were identical between C57BL and the wild-type mice regardless of slight different background of genes. In H_1KO and H_2KO mice, the increases in BPs produced by histamine were about a half of those in C57BL and wild-type mice. The decrease in HR produced by histamine in H_1KO mice was not significantly different from those in C57BL and wild-type mice, whereas the decrease in HR was almost absent in H_2KO mice. In DKO mice, the changes in both BPs and HR were abolished. Intrathecal administration of CSF did not produce significant changes in BPs or HR in any groups of mice (data not shown).

Selective stimulation of H_1, H_2 or H_3 receptor in C57BL mice.

The dose of each selective agonist was chosen as its peak effect. The H_1 selective agonist HTMT elicited a pressor response at a dose of 3.2 nmol; the rises in SBP, MBP, and DBP were 21.0 ± 2.5, 19.2 ± 1.8, and 18.1 ± 2.1 mmHg (n=6), whereas it did not produce any significant decrease in heart rate (-12.9 ± 15.5 bpm), when intrathecally administered to C57BL mice (Fig. 4A, B). The H_2 selective agonist dimaprit increased the blood pressure at a dose of 32 nmol and decreased the heart rate (-36.3 ± 8.3 bpm, n=6) when intrathecally administered to C57BL mice (Fig. 4A, B). The rises in SBP, MBP, and DBP were 20.1 ± 2.2, 17.1 ± 1.2, and 15.4 ± 1.2 mmHg, respectively. In contrast to the effects of histamine, HTMT and dimaprit, an H_3 selective agonist, immepip produced a slight but consistent decrease in BPs when administered intrathecally to C57BL mice at a dose of 3.2 nmol (Fig. 4A, B). The decreases in SBP, MBP, and DBP were 7.7 ± 0.9, 5.6 ± 0.4, and 4.6 ± 0.8 mm Hg, respectively, whereas HR was not changed (8.3 ± 7.1 bpm, n=6).

The changes induced by histamine or the H_1 selective agonist HTMT in C57BL mice were compared with those by histamine in H_2KO mice (Fig. 4C, D). The pressor responses to histamine in H_2KO mice were significantly smaller than those produced by histamine or HTMT in C57BL mice, whereas the decrease in HR by histamine in C57BL mice was abolished in other groups. The differences in the increases in BPs between them were around 7 mmHg. The changes induced by histamine or the H_2 selective agonist dimaprit in C57BL mice were compared with those by histamine in H_1KO mice (Fig. 4E, F). The decreases in HR were almost the same among three groups of mice, whereas the pressor response to histamine in H_1KO mice was significantly lower than those in other groups. The differences between them in BPs

were very similar to the decreases in BPs by the stimulation of H_3 receptor with immepip in C57BL mice.

The involvement of peripheral autonomic receptors in intrathecally administered histamine agonists-induced responses

The intraperitoneal injection of phenylephrine (4.9 nmol/g) produced an increase SBP from 100.9 ± 4.4 to 125.4 ± 6.1 mmHg (+24.5 ± 3.3 mmHg, n=10, $P<0.01$) and a decrease in HR from 537.3 ± 12.6 to 437.7 ± 9.6 bpm (-99.6 ± 15.7 bpm, n=10, $P<0.01$) 10 min after the administration in C57BL mice. The decrease in HR induced by peripherally acting phenylephrine was larger than that by intrathecally-administered 3.2 nmol histamine (-47.4 ± 5.0 bmp, n=6, $P<0.05$) in spite of the increase in SBP by histamine (+25.2 ± 5.0 mmHg) was same as that by phenylephrine.

The changes in SBP and HR induced by intrathecally-administered histamine were examined under the blockade of muscarinic receptors by atropine (i.p.). The blocking effect of atropine continued more than 60 min after the administration, which was evaluated by the increase in HR in the different experiments. HR was increased by atropine from 543.3 ± 28.7 to 619.5 ± 27.6 bpm (n=4, $P<0.01$), then atropine abolished the decrease in HR induced by histamine (632.9 ± 28.0). SBP before atropine, 30-min after atropine and about 15-min after histamine were 97.6 ± 4.7, 95.2 ± 7.5 and 124.6 ± 4.5 mmHg, respectively (n=4). The summarized data of histamine-induced responses in the absence and presence of the blockade of muscarinic receptors are presented in Fig. 5A and B. Although the increase in SBP by histamine (i.t.) was not changed, the

decrease in HR by histamine was completely abolished by atropine.

The changes in SBP and HR induced by intrathecally-administered dimaprit were examined under the blockade of adrenergic α_1 receptors by prazosin (i.p.). The blocking effect continued more than 60 min after the administration, which was evaluated by the decrease in SBP in the different experiments. SBP was decreased from 109.8 ± 5.3 to 94.3 ± 3.9 mmHg (n=6, $P<0.05$) after the administration of prazosin, then a slight increase in SBP was observed after the administration of dimaprit (104.9 ± 2.6 mmHg, $P<0.05$). HR before prazosin, 30-min after prazosin and about 15-min after dimaprit were 506.9 ± 20.1, 536.9 ± 23.5 and 494.9 ± 20.7 bpm, respectively (n=6). The summarized data of the responses to dimaprit in the absence and presence of the blockade of adrenergic α_1 receptors are presented in Fig. 5C and D. Although the increase in SBP by dimaprit (i.t.) was reduced by prazosin, the decrease in HR by dimaprit was not changed.

Discussion

Our major findings in this study are as follows; 1) intrathecally-administered histamine induced the pressor responses and bradycardiac effects in normal control mice including wild-type mice; 2) the pressor responses by histamine in H_2KO or H_1KO mice were about a half of those in normal control mice; 3) the bradycardiac effect of histamine was not different between H_1KO and its control mice, whereas histamine-induced bradycardia was almost abolished in H_2KO mice; 4) histamine did not change blood pressures and heart rate at all in double H_1 and H_2 receptor KO mice; 5) the selective stimulation of H_3 receptor by immepip generated a small but consistent depressor response in C57BL mice; 6) histamine-induced bradycardia which was abolished by atropine was much smaller than that induced by peripherally administered phenylephrine when compared with the same pressor responses.

This study elucidates the central nervous roles of the three histamine receptors on cardiovascular functions using the histamine receptors gene knockout mice and the specific agonists. The baseline BPs and HR in H_1KO, H_2KO and DKO mice were not different from those of their control mice. The intrathecally-administered histamine increased BPs and simultaneously decreased HR in mice, and the hypertensive effects were the essentially the same as those of intracerebroventricularly-administered histamine reported previously (Hicks 1978, Klein and Gertner 1981; Poulakos and Gertner 1989; Brown et al. 2001), although the bradycardiac effect in mice is different from the previous results with urethane-anesthetized rats (Finch and Hicks 1976; Hicks 1978). The influence of anesthesia on the effect of centrally-administered histamine on heart rate has been discussed (Poulakos and Gertner 1989; Brown et al. 2001). Thus, the

tail cuff method (Murakami et al. 2000; 2003; Hagiwara et al. 2003) to measure blood pressure and heart rate in conscious mice gives reliable results as direct intra-arterial catheter method (Krege et al. 1995). Furthermore, the intrathecal injections of histamine receptor agonists in mice produce the equivalent results induced by the intracerebroventricular administration in rat (Poulakos and Gertner 1989). In the examination of the contribution of histamine receptor subtypes to cardiovascular effects, histamine antagonists may have some cardiovascular effects unrelated to blocking the histamine receptors (Gatti and Gertner 1984). For example, the possible involvements of putative imidazole binding sites seem blunted the analysis and explanation of the results with H_2 antagonists (Poulakos and Gertner 1989). The application of the inhibitor of histamine-N-methyltransferase revealed the roles of the endogenous histamine in central cardiovascular regulation (Klein and Gertner 1981), while little information on its receptor subtypes was obtained. Furthermore, the levels of inverse agonism of different blockers against histamine receptors in various systems also affect the results, especially *in vivo*. Thus, we have chosen the use of the selective agonists and histamine receptor gene knockout mice in this study.

The selective central stimulation of H_1 receptor by the H_1 agonist HTMT in C57BL mice produced a pressor response without changes in HR. These finding confirmed the observations of Poulakos and Gertner (1989) where pyridylethylamine as an H_1 agonist was administered into lateral ventricle (i.c.v.) of conscious rats. The selective central stimulation of H_2 receptor by the H_2 agonist, dimaprit, in C57BL mice produced a pressor response with a decrease in HR, which results are also consistent with those of Poulakos and Gertner (1989) where impromidine was used as an H_2 agonist in conscious rats. Although the decreases in HR in C57BL, wild-type, and H_1KO mice

were almost the same, the increase in BPs in H_1KO mice were low. The decrease in HR in C57BL mice was abolished by the muscarinic blocker, atropine, indicating central vagal nerve activity is enhanced by H_2 receptors. The peripherally pressor response of phenylephrine induced more profound reflex bradycardia compared with the decrease in HR by central histamine. The bradycardiac response to central dimaprit was not changed by prazosin which reduced the increase in SBP by dimaprit. These results indicate the decrease in HR is not due to the reflex bradycardia induced the pressor response to centrally administered histamine but mainly due to the direct stimulation of central H_2 receptors which drive vagal output to the heart *via* nucleus dorsalis nervi vagi and/or nucleus ambiguus. The involvement of H_2 receptor in bradycardiac responses to histamine and the selective H_2 receptor agonist in this study shows the independent central regulation of HR and vascular resistance (*via* vasomotor center of rostral ventral lateral medulla) by central histaminergic nerves, as shown previously (Finch and Hicks 1976). In accordance with our findings, peripheral hyperosmolality significantly increased MBP, but reactive bradycardia was abolished after i.c.v. administration of H_2 receptor antagonists in conscious rats (Kenney and Bealer 1993). The prazosin-resistant pressor response to dimaprit may be partly due to the hypothalamic stimulation resulting in the release of pressor agents like vasopressin (Bealer 1993; Bealer and Abell 1995; Knigge et al. 1999).

The depressor response by H_3 receptor may be due to the stimulation of the autoreceptors on central histaminergic nerve fibers, since no change in blood pressure was observed in DKO mice by the administration of histamine, in which H_3 receptor would be stimulated. If there were H_3 receptors as the heteroreceptors on the different nerves from histaminergic one related with the central regulation cardiovascular system,

like dopaminergic nerve in the striatum (Göthert et al. 1995), the stimulation H_3 receptor by histamine in DKO mice would have produced a depressor response. The autoregulated release of histamine via H_3 receptors has been reported in medulla oblongata of rabbit where H_3 receptors have an important role to control the tonic output of histamine (Kanamaru et al. 1998). The spontaneous firing of a tuberomamillary neuron *in vivo* was reduced by an H_3 inverse agonist, thioperamide, indicating a tonic inhibitory H_3 autoreceptors in histaminergic system (Haas and Panula 2003). The finding of the depressor effect of the central stimulation of H_3 autoreceptors in mice is a new finding to our knowledge. The central histaminergic nerve activity will be beneficial mechanism for surviving from severe hypotension, when the pressor response with bradycardia or suppression severe tachycardia is required. In anesthetized rats with hypovolemic shock, the increase in central histamine by inhibiting histamine N-methyltransferase resulted in an increase in the survival rate (Jochem 2002; 2004).

Conclusion

In this study using histamine H_1 and/or H_2 receptors gene knockout mice, we have demonstrated that the pressor responses to the centrally-administrated histamine are mediated through the stimulation of both H_1 and H_2 receptors, whereas the decrease in heart rate is mainly due to the functions of H_2 receptor which activate the vagal output to the heart. The selective central stimulation of H_3 receptor produces a sight but consistent decrease in blood pressure via autoreceptors in histaminergic nerves. The combination of the gene targeting techniques with selective agonists or blockers is thus informative, consistent, and decisive. Further studies using histamine related gene knockout mice (Inoue et al. 1996, Kobayashi et al. 2000, Ohtsu et al. 2001, Toyota et al. 2002) are needed to clarify the precise roles of histamine and its receptors on cardiovascular functions.

Acknowledgements: This work was supported in part by Grants-in-Aid for scientific research from the Japan Society of Promotion of Science (JSPS) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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Fig.1 A, Baseline systolic blood pressure (SBP) in C57BL, wild-type, H_1 receptor gene knockout (H_1KO), H_2 receptor gene knockout (H_2KO), and H_1 and H_2 receptor gene double knockout (DKO) mice. **B**, Baseline heart rate (HR) in C57BL, wild-type, H_1KO, H_2KO, and DKO mice. N = 69 in C57BL, n=40 in wild-type, n = 46 in H_1KO, n = 37 in H_2KO, and n = 16 in DKO mice.

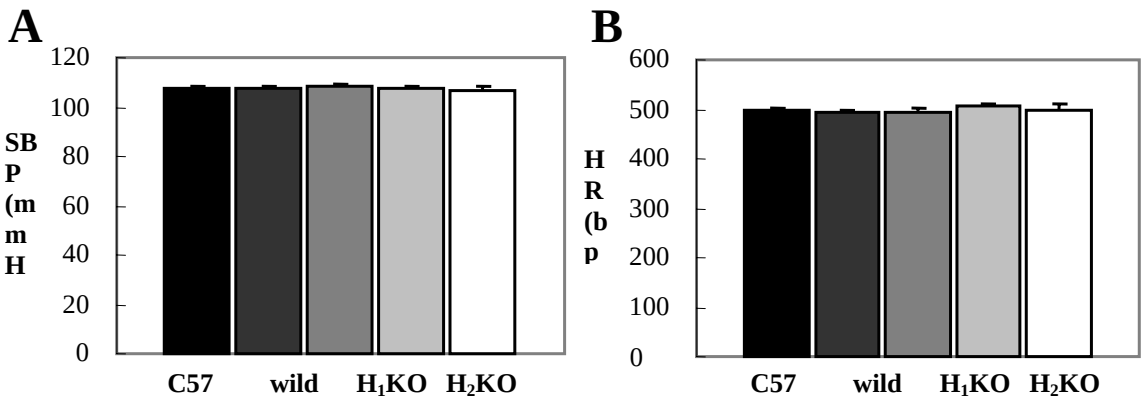
Fig. 2 A, B. The time course of the responses of systolic blood pressure (SBP) and heart rate (HR) to 3.2 nmol histamine given intrathecally to C57BL mice (n=6). **C, D.** Effects on SBP and HR of 1.0, 3.2, and 10 nmol histamine given intrathecally to C57BL mice. N = 6 in each doses.

Fig. 3 Effects of 3.2 nmol systolic blood pressure (SBP, **A**) and histamine on heart rate (HR, **B**) when intrathecally administered to C57BL, wild-type, H_1KO, H_2KO, and DKO mice. N = 6 in each mice group. The significance of differences to C57 was compared by a two-way analysis of variance and Scheffe test. (*P < 0.05; **P < 0.01; ***P < 0.001 vs. C57BL).

Fig. 4 Selective stimulation of H_1, H_2 or H_3 receptor in C57BL mice. **A, B** Effects of the H_1 selective agonist HTMT (3.2 nmol), the H_2 selective agonist dimaprit (32 nmol), and the H_3 agonist immepip (3.2 nmol) on systolic blood pressure (SBP) and heart rate (HR) intrathecally given to C57BL mice (n = 6, *P < 0.05 vs. baseline). The changes in SBP by three selective agonists were statistically significant, though the decrease in SBP by H_3 agonist immepip was slight. **C, D.** Effects on SBP and HR of intrathecally-administered histamine (3.2 nmol, n=6) or HTMT (3.2 nmol, n=6) in C57BL mice and histamine (3.2 nmol) in H_2KO mice (n=6; *P < 0.05, **P < 0.01 vs. histamine; ^{b}P<0.01 vs. HTMT). **E, F.** Effects on SBP and HR of histamine (3.2 nmol, n=6) or dimaprit (32 nmol, n=6) in C57BL mice and histamine (3.2 nmol) in H_1KO mice (n=6; *P < 0.05 vs. histamine; ^{a}P<0.05 vs. dimaprit).

Fig. 5 A, B The influence of atropine (i.p., 1.4 nmol/g) on changes in SBP and HR induced by intrathecally-administered histamine (***P < 0.001 vs. histamine alone). **C, D** The influence of prazosin (i.p., 0.72 nmol/g) on changes in SBP and HR induced by intrathecally-administered dimaprit (*P <0.05 vs. dimaprit alone).

Fig. 1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60
40
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SBP (mmH
C57
wild
H1KO
H2KO
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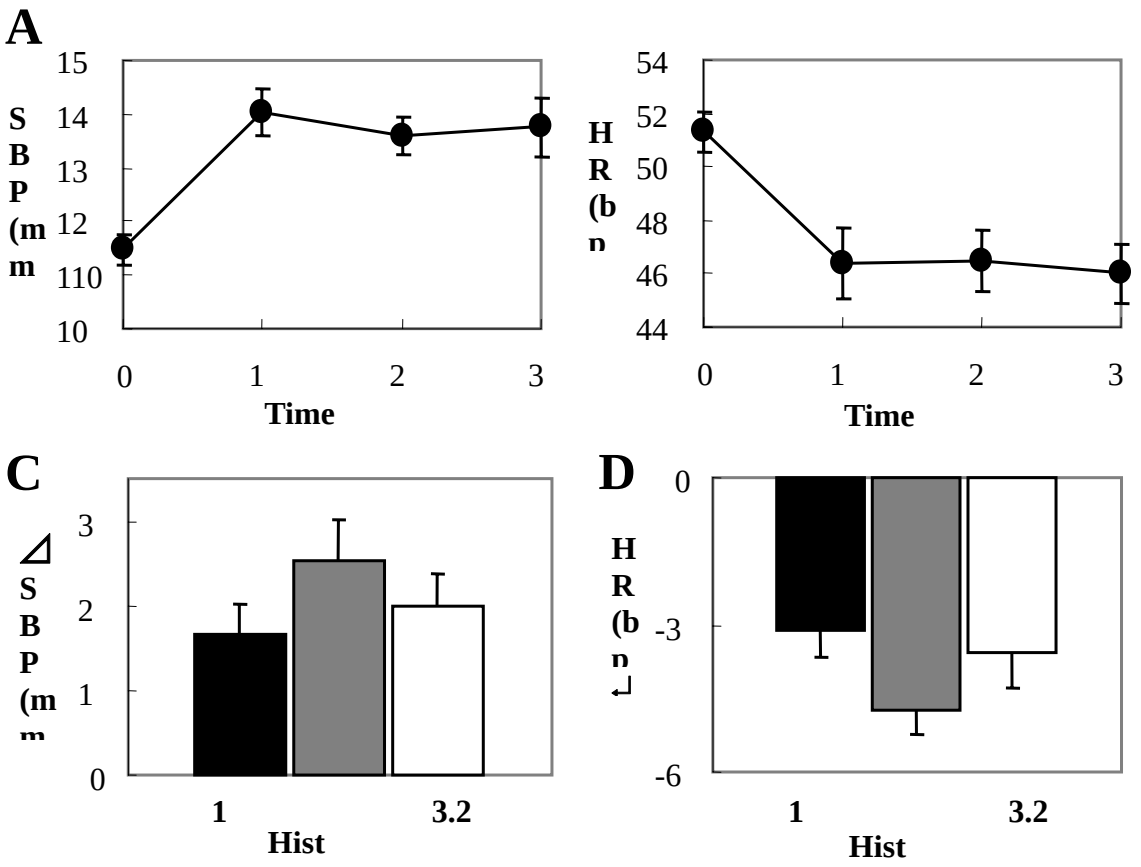
400
300
200
100
0
HR (bp
C57
wild
H1KO
H2KO

Fig. 2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12
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10
0
1
2
3
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HR (bp
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52
50
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46
44
0
1
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3
Time
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⊿SBP (mm
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1
3.2
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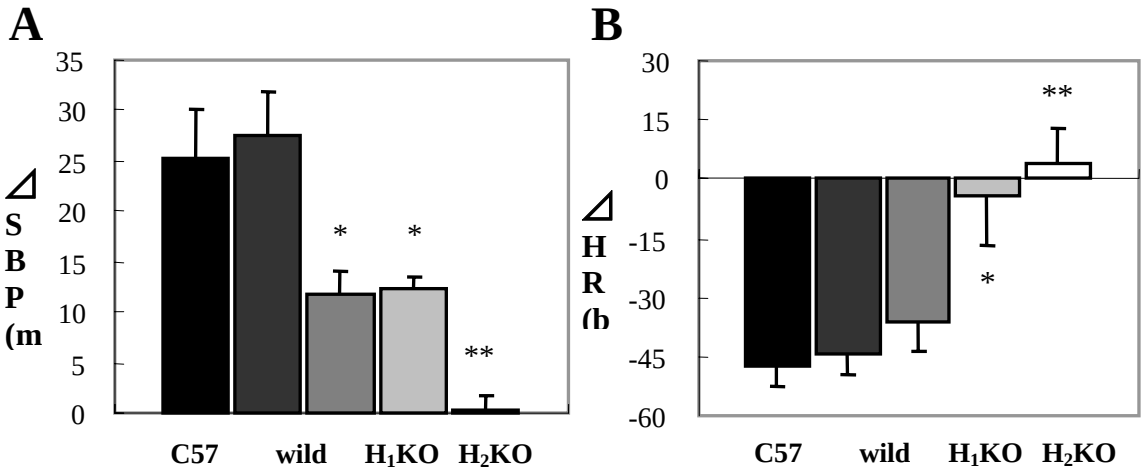
HR (bp
0
-3
-6
1
3.2
Hist

Fig. 3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20
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10
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0
*
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**
C57
wild
H1KO
H2KO
B
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0
-15
-30
-45
-60
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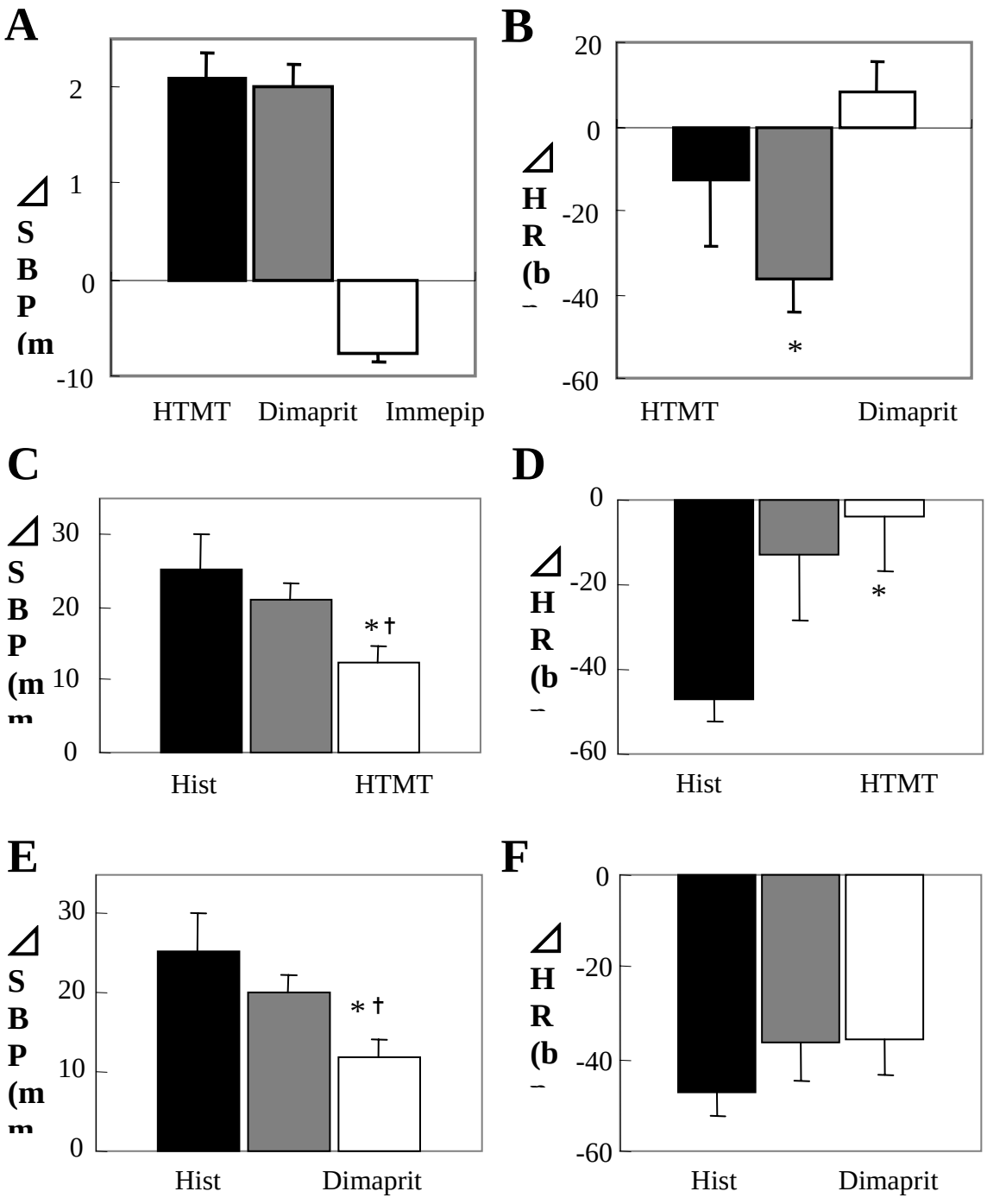
*
C57
wild
H1KO
H2KO

Fig. 4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20
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Hist
HTMT
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Hist
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E
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Hist
Dimaprit
F
0
-20
-40
-60
Hist
Dimaprit

Fig. 5