Blood Pressure and Heart Rate Are Increased by AF-DX 116, a Selective $M_2$ Antagonist, in Autonomic Imbalanced and Hypotensive Rats Caused by Repeated Cold Stress

Taeko Hata$^1$, Eiji Itoh$^1$, Yoshinori Funakami$^1$, Katsushi Ishida$^1$ and Shuji Uchida$^2$

$^1$Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kinki University, 3-4-1 Kowakae, Higashi-Osaka 577-8502, Japan
$^2$Kawanishi Pharma Research Institute, Nippon Boehringer Ingelheim Co., Ltd., 3-10-1 Yatoi, Kawanishi 666-0131, Japan

Received April 12, 2000 Accepted December 21, 2000

ABSTRACT—Rats exposed to SART (specific alternation of rhythm in temperature) stress, which are ideal animal models for vagotonia-type dysautonomia, show various changes in cardiac and circulatory systems. In this study, attention was directed to cholinergic function in the SART-stressed rat heart and the effects of AF-DX 116, a specific muscarinic $M_2$ antagonist, on blood pressure and heart rate. The results were compared with those obtained for atropine and pirenzepine. In SART-stressed rats, systolic and diastolic blood pressures (SBP and DBP) were lower than in unstressed rats. Oral AF-DX 116 resulted in greater elevation of DBP than SBP in unstressed rats. In stressed rats, greater and more prolonged elevation of SBP than in unstressed rats was noted, particularly at higher doses. A dose-dependent SBP change in stressed rats, caused by intravenous AF-DX 116, was shifted upward in parallel with that in unstressed groups, unlike with oral administration. The positive chronotropic effect of this drug was smaller in stressed rats than in unstressed rats, in contrast to the pressor effect. SART-stressed rats may thus have an enhanced sympathetic tone in the heart, as well as changes in muscarinic $M_2$ receptors at sympathetic nerve endings and at the heart muscle. The effects of AF-DX 116 on blood pressure and heart rate thus may arise from peripheral action and AF-DX 116 may be useful for treating hypotension related to autonomic imbalance of the vagotonia type.

Keywords: Stress, Blood pressure, AF-DX 116, Hypotension, $M_2$ antagonist

Blood pressure (BP) is related to sympathetic tone and physical and/or psychological stress (1 – 7). In general, stress is thought to induce hypertension in humans and experimental animals. Rats subjected to SART (specific alternation of rhythm in environmental temperature) stress (8) showed hypotension (9) in a manner different from hypertension in animals subjected to other types of stress such as cold and restraint (10 – 12).

SART stress is induced through sudden drops in environmental temperature and is a type of repeated or intermittent cold stress in rodents. Experimental animals subjected to SART stress are accepted as animal models of vagotonia-type dysautonomia in consideration of the results of Aschner and Mecholyl tests (13). The stressed animals exhibit pathophysiological abnormalities such as an abnormal galvanic skin response (GSR) (8) and electroencephalogram (EEG) (14), hyperalgesia (15), thrombocytopenia (16), as well as hypotension (9). In cardiac and circulatory systems, the animals show tachycardia, short PQ and long QRS intervals and high voltage in R waves on electrocardiograms (ECG) (17), and blood flow either increases or decreases in the arteries (9, 18). In the cholinergic system, a decrease in acetylcholine (ACh) content and an elevation of the synthesis and hydrolysis of ACh in the brain (19) were noted in addition to an increase in ACh content, a decrease in ACh-esterase activity and the number of muscarinic ACh receptors in the small intestine (20).

SART-stressed animals show high parasympathetic nerve activity, but low sympathetic nerve activity (13), possibly resulting in lower blood pressure and an abnormal ECG. Thus, cholinergic function in regard to BP and heart rate (HR) of SART-stressed rats was investigated using muscarinic antagonists, especially AF-DX 116, a specific muscarinic $M_2$ antagonist, in this study. AF-DX 116 has already been reported to increase BP and HR in rats and dogs (21, 22). The effects of AF-DX 116 on BP and HR in SART-stressed rats were compared with those of other muscarinic antagonists, pirenzepine, an $M_1$ specific antagonist, and atropine, a nonselective muscarinic antagonist.
MATERIALS AND METHODS

Animals and stress loading

Animals: Male Wistar rats (Japan SLC Inc., Hamamatsu) weighing 250 to 300 g were kept in a climate-controlled room with a temperature of 24 ± 1°C and a relative humidity of 55 ± 15% on a 12-h light-dark cycle (lights on at 07:00 h, off at 19:00 h) with access to a standard diet (MF; Oriental Yeast, Tokyo) and tap water ad libitum.

Loading SART stress: Rats were stressed essentially as previously reported (23). Rats were alternately transferred to two cages, one placed in a room at 24°C and the other in a room at −3°C, every hour from 09:00 h to 16:00 h and housed in a cage at −3°C from 16:00 h to 09:00 h the following morning. This procedure was repeated for 5 – 7 consecutive days up to 11:00 h on the day of the experiment. The relative humidity conditions were 55 ± 15% in the former room and 85 ± 15% in the latter room. The stressed rats were kept at room temperature (24°C) for at least 30 min before the experiment.

This study was conducted in accordance with ethical procedures approved for the care and use of laboratory animals by The Japanese Pharmacological Society.

Drugs

The following anticholinergic drugs were used: AF-DX 116 (Nippon Boehringer Ingelheim, Kawanishi), atropine sulfate (Wako Pure Chemical Industries, Osaka) and pirenzepine dihydrochloride (Research Biochemicals, Inc., Natick, MA, USA). AF-DX 116 was dissolved in 0.1 N HCl, adjusted to pH 7.4 by 0.1 N NaOH and a concentration of 1% by physiological saline, and stored in a refrigerator. Atropine and pirenzepine were dissolved in physiological saline. All drug solutions were prepared at fixed volumes of 1.0 ml/kg body weight immediately before use and administered orally or into the tail vein.

Measurement of blood pressure and heart rate

Indirect BP and HR were measured by using an automatic blood pressure meter (BP-98A; Softron, Tokyo) following the tail-cuff method. Briefly, a photoelectric sensor, which is attached to the tail by a piece of rubber tubing, detects pulse volume oscillation by cuff-pressure and pulse waves. Pressures with the re-flow of blood and at maximal amplitude of pulse waves are detected as systolic blood pressure (SBP) and mean blood pressure (MBP), and these values are used to calculate diastolic blood pressure (DBP) and HR automatically [DBP = (3 × MBP – SBP) / 2]. Before measurement, the rats were warmed for about 5 min in a box controlled at 40°C to facilitate detection of the pulse of the tail artery.

Statistical analyses

All data were expressed as means ± S.E.M. Data for 2 groups were analyzed by Student’s t-test and data for multiple groups by Tukey’s test and one-way or two-way ANOVA. P<0.05 was considered significant.

Dose-response lines were drawn based on least-squares analysis results.

RESULTS

Hypotension in SART-stressed rats

SBP was about 130 mmHg in unstressed rats. Under SART-stress conditions, SBP dropped gradually for 5 – 7 days until reaching a plateau at 10 mmHg less than that of unstressed rats, as reported previously (9). DBP changed in a manner similar to SBP.

SBP, MBP and DBP values of unstressed and SART-stressed rats are shown in Table 1. SBP of SART-stressed rats was significantly less than that of unstressed rats, as were also MBP and DBP. The decreases in DBP and MBP in stressed rats were basically the same as that in SBP. Pulse pressure, determined from SBP and DBP was less in stressed rats than in unstressed rats. The ratio of data in stressed rats (S) to data in unstressed rats (N) (S/N ratio) was 0.87 – 0.92, being the same in all indexes. SBP and DBP were thus used as indexes of drug effects in the following data.

Pressor effect of AF-DX 116

BP change with oral AF-DX 116: The time course of changes in blood pressure (ΔBP) after oral administration of AF-DX 116 is shown in Fig.1 for unstressed and SART-stressed rats. Oral AF-DX 116 enhanced BP dose-dependently for the most part in unstressed rats [P<0.01, F(2,63) = 12.978 for SBP, 26.063 for DBP] and in stressed rats [P<0.01, F(2,63) = 67.570 for SBP, 29.425 for DBP]. The effect of AF-DX 116 was maximal at about 90 min after oral administration in all cases.

ΔSBP in stressed rats was much greater than in unstressed rats [P<0.01, F(1,56) = 80.667 for 12 mg/kg and

| Table 1. | SART stress-induced changes in rat blood pressure |
|----------|-----------------------------------------------|
|          | No stress (N) | SART stress (S) | Ratio (S/N) |
| SBP (mmHg) | 130.5 ± 0.8 | 118.2 ± 1.4*** | 0.91 |
| MBP (mmHg) | 103.3 ± 1.0 | 100.1 ± 1.8*** | 0.91 |
| DBP (mmHg) | 98.8 ± 1.1 | 90.7 ± 2.0** | 0.92 |
| Pulse pressure (mmHg) | 31.8 ± 1.0 | 27.7 ± 1.0* | 0.87 |

The values represent the means ± S.E.M. of 12 rats. *P<0.05, **P<0.01 and ***P<0.001, significantly different from values of unstressed rats (Student’s t-test).
Stress-Induced Hypotension and AF-DX 116

F(1,42) = 183.115 for 24 mg/kg], except for groups treated with 6 mg/kg of AF-DX 116 [F(1,28) = 0.133], and high values were still evident even 4 h after drug administration, although ΔSBP in unstressed rats was nearly zero at 3 h. The time course of ΔDBP was basically the same for both stressed and unstressed rats at 6 mg/kg [F(1,28) = 0.133], 12 mg/kg [F(1,56) = 0.254] and 24 mg/kg [F(1,42) = 1.316] of oral AF-DX 116 by two-way ANOVA.

ΔBP at 90 min after oral administration, during which time it was almost maximal, is shown in Fig. 2. In unstressed rats, ΔSBP slightly increased by the administration of 6–24 mg/kg of AF-DX 116. ΔDBP increased dose-dependently (P<0.05), significantly exceeding ΔSBP (P<0.01). In SART-stressed rats, oral AF-DX 116 elevated SBP (P<0.01) and DBP (P<0.05) dose-dependently and significantly. Two-way ANOVA applied to dose-dependent changes in ΔBP showed significant differences in ΔSBP for both stressed and unstressed rats [P<0.01, F(1,18) = 32.532]. ΔDBP was essentially the same in the two groups, although dose-dependent changes were evident.

Time-dependent changes in ΔBP with the administration of 24 mg/kg of AF-DX 116 and 40 mg/kg of atropine are shown in Fig. 3. Each drug at this dose caused a similar elevation of SBP in unstressed rats. Atropine increased ΔSBP and ΔDBP basically to the same extent in stressed and unstressed rats. The pressor effect of AF-DX 116 in unstressed rats was similar to that of atropine in SBP [F(1,61) = 0.342] but greater [P<0.01, F(1,61) = 25.832] than that of atropine in DBP. In SART-stressed rats, the effect of AF-DX 116 was stronger than that of atropine on SBP and DBP [P<0.01, F(1,64) = 260.457 for SBP, 53.187

Fig. 1. Time course of blood pressure changes in rats after oral administration of AF-DX 116. Each point represents the mean ± S.E.M. of 3 to 5 unstressed (○, △, □) and SART-stressed (●, ▲, ■) rats. AF-DX 116 was orally administered at 24 mg/kg (○, ●), 12 mg/kg (△, ▲) and 6 mg/kg (□, ■). **P<0.01, significantly different in the two groups (two-way ANOVA).

Fig. 2. AF-DX 116-induced increase in blood pressure of unstressed and SART-stressed rats. The values represent the means ± S.E.M. of 3 to 5 unstressed (□) or SART-stressed (■) rats, 90 min after oral administration of AF-DX 116. **P<0.01, significantly different in unstressed and SART-stressed groups (two-way ANOVA).
for DBP]. Atropine caused small decreases in BP 30 min after administration in unstressed and stressed rats, but AF-DX 116 increased BPs from 30 min after administration without any drop in the early stage.

Dose-response lines, which are shown in Fig. 4 relating to ΔBPs caused by AF-DX 116 and atropine, represent the means of the ΔBPs at the peaks of the time-dependent curves shown in Fig. 1 (primarily the means of the two highest values at 30 – 90 min). The ΔSBP lines for atropine in stressed and unstressed rats were the same, as were also the ΔDBP lines. For AF-DX 116, the ΔSBP line for unstressed rats was similar to that induced by atropine, but that for stressed rats shifted upward non-parallelly with that for unstressed rats. ΔDBP lines for AF-DX 116 were the same for stressed and unstressed rats, parallel to and situated above the atropine lines.

Effects of intravenous AF-DX 116: The augmenting effects on BP when AF-DX 116 was administered intravenously appeared to predominate in the early phase, compared to oral administration of AF-DX 116, and they were maximum at 30 – 60 min. Dose-response lines of ΔBPs at the peak time after intravenous AF-DX 116 administration are shown in Fig. 5. Intravenous AF-DX 116 increased SBP in unstressed rats more prominently and with clear dose-dependency, compared to oral administration of this drug. The ΔSBP and ΔDBP values of stressed rats exceeded these values for unstressed rats \([P<0.01, F(1,16) = 25.03 \text{ for } \text{SBP}; P<0.05, F(1,16) = 4.397 \text{ for } \text{DBP}].\) The ΔBP lines for stressed rats shifted upward, paralleling those for unstressed rats, in contrast to ΔSBP lines after oral administration.

The time-related changes in ΔBPs induced by 100 µg/kg of AF-DX 116 and 40 µg/kg of atropine, which caused similar maximal SBP values in unstressed rats, are presented in Fig. 6. The ΔBPs induced by AF-DX 116 had no early negative phase, in contrast to changes caused by atropine. ΔBPs of SART-stressed rats with intravenous AF-DX 116 exceeded those with atropine \([P<0.01, F(1,42) = 18.840 \text{ for SBP, 7.916 for DBP}],\) although not as remarkably as in oral administration.

The ΔBP values recorded after intravenous AF-DX 116, atropine and pirenzepine at the respective peak times are shown in Table 2. AF-DX 116 dose-dependently increased both ΔSBP and ΔDBP in the two groups. The effects were significantly greater in the stressed rats than in the unstressed rats by two-way ANOVA \([P<0.01, F(1,16) = 25.030 \text{ for } \text{SBP}; P<0.05, F(1,16) = 6.576 \text{ for } \text{DBP}].\) The pressor effects of atropine and pirenzepine were small and essentially the same in the groups; there were no significant differences between the stressed and unstressed groups \([F(1,12) = 1.833 \text{ and } 0.818 \text{ for atropine in SBP and DBP; } \text{F}(1,14) = 0.312 \text{ and } 0.167 \text{ for pirenzepine in SBP and } \text{DBP}].\)
Stress-Induced Hypotension and AF-DX 116

DBP]. These effects were not dose-dependent in most of the groups, except for ΔSBPs in unstressed groups treated with 20 and 40 μg/kg of atropine.

Effects of AF-DX 116 on HR

The basal HR of SART-stressed rats was 375.8 ± 2.7 beats/min, and it was significantly higher (P<0.01) than that of unstressed rats, 342.2 ± 4.3 beats/min. As shown in Fig. 7, AF-DX 116 administered intravenously increased HR, and ΔHR rose dose-dependently in the two groups. The chronotropic effect of AF-DX 116 was 0.4 – 0.5 times smaller in the stressed rats than in the unstressed rats. No significant differences could be detected in the effects of atropine and pirenzepine on the two groups, although definite chronotropic effects were apparent.

DISCUSSION

The relation of stress to BP has long been studied. Stress-induced hypertension in humans and animals, such as immobilization-induced elevation in SBP (12), hypertension caused by social interactions and/or physiological stress (2, 6) in rats and the development of hypertension in borderline-hypertensive rats (3, 5, 24, 25) subjected to restraint and conflict have come under frequent consideration.

Stress-induced hypotension (26 – 29) has been little studied. Rats exposed to SART stress showed lower SBP than unstressed rats by 10 – 20 mmHg, as reported previously (9). DBP of SART-stressed rats was first observed in this study to be lower than that of unstressed rats to the same extent as for SBP. Thus, SART stress causes hypotension with the same decreases in both SBP and DBP of rats.

SART stress caused tachycardia in rats and mice, as also noted previously (17). Many factors contribute to the regulation of BP and HR, such as, significantly, autonomic nervous activity. Restraint stress usually induces an increase in HR and BP in rats. However, demedullated and chemically sympathectomized rats are reported to show some increase in HR and a decrease in BP caused by stress (4). The restraint stress-induced increase in HR may be related to sympathetic nerve activity and the increase in BP to adrenal function. But contrary to the previous studies, attention here was directed to cholinergic function in the SART-
stressed rat heart and the effects of anticholinergic drugs, especially the muscarinic M₂ antagonist, AF-DX 116, on hypotension.

The muscarinic M₂ receptor is a subtype with selective affinity toward heart muscle (30, 31). Anticholinergic drugs exert a positive chronotropic effect on the heart subsequent to an increase in parasympathetic nerve activity. Intravenous AF-DX 116 increased HR and BP in conscious dogs dose-dependently, and the pressor action was selective toward DBP, not SBP (K. Thomae et al., unpublished proprietary data). The pressor action of oral and intravenous AF-DX 116 in unstressed rats in this study was much greater on DBP than SBP, as in dogs. The dose-response lines of DBP shifted upward relative to those with atropine in unstressed rats, although the SBP curve was similar to the curve for atropine. These differential effects of AF-DX 116 were emphasized in SART-stressed rats, as shown in Fig. 6.

Fig. 6. Comparison between effects on blood pressure of intravenous AF-DX 116 and atropine in unstressed and stressed rats. The points represent the means ± S.E.M. of 4 or 5 unstressed (○, △) and SART-stressed (●, ■) rats administered intravenous AF-DX 116 (100 µg/kg) (○, ●) or atropine (40 µg/kg) (△, ■).

### Table 2. Increase in blood pressure due to muscarinic antagonists in unstressed and SART-stressed rats

| Drug        | Dose (µg/kg) | ΔSBP (mmHg) No stress | ΔSBP (mmHg) SART stress | ΔDBP (mmHg) No stress | ΔDBP (mmHg) SART stress |
|-------------|--------------|------------------------|-------------------------|-----------------------|-------------------------|
| AF-DX 116   | 25           | 3.67 ± 0.88            | 6.00 ± 0.00             | 6.50 ± 0.50           | 9.75 ± 0.95             |
|             | 50           | 2.67 ± 0.88            | 6.00 ± 0.00             | 6.00 ± 1.44           | 7.00 ± 1.53             |
|             | 100          | 7.50 ± 0.87            | 12.25 ± 1.31            | 6.50 ± 0.50           | 7.75 ± 0.95             |
|             | 200          | 10.25 ± 1.31           | 15.00 ± 0.82            | 6.00 ± 1.44           | 9.75 ± 0.95             |
| Atropine    | 20           | 3.67 ± 0.88            | 5.00 ± 0.58             | 3.00 ± 1.00           | 4.00 ± 1.15             |
|             | 40           | 7.60 ± 0.68            | 9.00 ± 1.22             | 3.60 ± 1.29           | 5.00 ± 1.26             |
| Pirenzepine | 200          | 1.33 ± 0.17            | 2.17 ± 0.67             | 2.00 ± 0.76           | 3.67 ± 1.30             |
|             | 400          | 2.83 ± 0.93            | 3.00 ± 2.89             | 4.17 ± 1.20           | 4.33 ± 0.83             |
|             | 800          | 3.83 ± 0.37            | 4.42 ± 0.37             | 5.21 ± 0.90           | 4.29 ± 0.44             |

The values represent means ± S.E.M. of 3 or 4 rats. Drug effects were observed at the respective peak time after i.v. administration. Significant differences between unstressed and SART-stressed groups were observed only in AF-DX 116-treated rats at $P<0.01$ for ΔSBP and at $P<0.05$ for ΔDBP (two-way ANOVA).
DX 116 on SBP and DBP may possibly arise from peripheral action and not central action, which must be the same for both SBP and DBP. AF-DX 116 did not cause a transient drop in BP soon after its administration; atropine showed a drop within 15–30 min after administration not only in unstressed rats, but also in SART-stressed rats. This may be characteristic of AF-DX 116, in contrast to atropine.

In SART-stressed rats, the AF-DX 116 action on DBP was the same as and paralleled that in unstressed rats, but its effect on SBP was much greater, especially at higher doses, compared to its effect in unstressed rats. The dose-response curve shifted to the left and upward in a nonparallel fashion to the curve in unstressed rats, particularly at higher doses. Atropine had the same action on SBP and DBP of SART-stressed rats as on these values for unstressed rats. Pirenzepine, a selective M₁ antagonist, had the same very weak action on both SBP and DBP in unstressed and SART-stressed rats. SART-stressed rats show increased parasympathetic activity (17), resulting from the small release of norepinephrine at sympathetic nerve endings in the heart. The considerable effect of AF-DX 116 on SBP in SART-stressed rats may result from the increase in cardiac output induced by a greater norepinephrine release at sympathetic nerve endings through blockage of M₂ receptors by AF-DX 116 at sites in the heart and from the antagonistic effect on negatively inotropic action through M₂ receptors in the ventricular myocardium. Atropine and pirenzepine most likely decrease arterial blood pressure through central muscarinic M₁ cholinoreceptors (32, 33). In this study, atropine may act through M₂ receptors at the same peripheral sites in a manner similar to AF-DX 116, but have a greater effect through the central muscarinic M₁ receptors.

AF-DX 116 has a progressively greater effect on HR in healthy volunteers (34). In this study, AF-DX 116 caused a dose-dependent increase in HR, which was smaller in SART-stressed rats than in unstressed rats, in contrast to atropine and pirenzepine. HR is partially controlled through muscarinic receptors (except for the M₂ subtype) in the sinoatrial node, postganglionic M₁ receptors of the cardiac sympathetic nerves (35) and M₂ receptors at sympathetic nerve endings and in heart muscle (34). The increase in HR caused by AF-DX 116 may be smaller in SART-stressed rats, since the M₂ receptor may change and/or M₂ receptors may be less sensitive at the sinoatrial area and adjacent atrial myocardium, resulting in tachycardia in the stressed rats with increased parasympathetic activity in the heart (17). The effects of atropine on HR may arise primarily through the central muscarinic receptors rather than through peripheral M₂ receptors.

These drug effects on unstressed and SART-stressed rats thus differ only in AF-DX 116-treated groups, possibly since AF-DX 116 acts selectively through the muscarinic M₁ receptor. SART stress may also induce changes in peripheral M₂ receptors, such as subsensitivity in heart muscle and/or high sensitivity at sympathetic nerve endings in the heart, which results in tachycardia. The effects of atropine on the heart may be greater through central muscarinic receptors than through M₂ receptors in the sinoatrial area, adjacent atrial myocardium and sympathetic nerve endings in the heart, although detailed mechanisms must be pursued in further studies.

Regarding the muscarinic inhibition in heart muscle, the
characteristics of accentuated antagonism are known (36–39): vagal activity is predominant in the presence of strong background sympathetic activity and muscarinic inhibitory action is remarkable in heart muscle with elevated sympathetic tone, although the action is not as strong as in the normal heart. Ach has a negative inotropic effect in addition to a negative chronotropic action and depresses norepinephrine release at adrenergic nerve terminals in the cardiovascular system via presynaptic muscarinic receptors (36, 40). The positive inotropic action of AF-DX 116 is inhibited by propranolol, a β-adrenergic blocker (41). The strong positive inotropic action of AF-DX 116 in SART-stressed rats may thus be caused by an increased contraction of heart muscle due to norepinephrine release through blockage of M2 receptors at sympathetic nerve terminals.

This study thus suggests that sympathetic tone is enhanced in the heart and the number and/or affinity of the muscarinic M2 receptors may increase in the sympathetic nerve endings and decrease in the heart muscle of SART-stressed rats. The effects of AF-DX 116, an M2 antagonist, were found to be stronger in SART-stressed rats with changes in muscarinic M2 receptors, than in unstressed rats. AF-DX 116 should thus prove useful for treating hypertension related to autonomic imbalance of the vagotonia type.

REFERENCES

1 Sapira JD, Shapiro AP, Klainicki T, Yevick JE and Small JL: Studies in man on the relationship of adrenergic correlates to pressor responsiveness. Circulation 34, 226 – 241 (1966)
2 Fokkema DS and Kooolhaas JM: Acute and conditioned blood pressure changes in relation to social and psychosocial stimuli in rats. Physiol Behav 34, 33 – 38 (1985)
3 Lawler JE, Cox RH, Sanders BJ and Mitchell VP: The borderline hypertensive rat: a model for studying the mechanisms of environmentally induced hypertension. Health Psychol 7, 137 – 147 (1988)
4 Barron BA and Van Loon GR: Role of sympathoadrenomedullary system in cardiovascular response to stress in rats. J Auton Nerv Syst 28, 179 – 187 (1989)
5 Lawler JE, Sanders BJ, Cox RH, Mitchell VP and Baer PG: Bilateral renal denervation can prevent the development of stress-induced hypertension in the borderline hypertensive rat. Clin Exp Hypertens A 11, 1549 – 1563 (1989)
6 Henry JP, Liu YY, Nadra WE, Qian CG, Mormede P, Lemaire V, Ely D and Hendley ED: Psychosocial stress can induce chronic hypertension in normotensive strains of rats. Hypertension 21, 714 – 723 (1993)
7 Kanayama N, Tsujimura R, She L, Maehara K and Terao T: Cold-induced stress stimulates the sympathetic nervous system, causing hypertension and proteinuria in rats. J Hypertens 15, 383 – 389 (1997)
8 Kita T, Hata T, Yoneda R and Okage T: Stress state caused by alternation of rhythm in environmental temperature, and the functional disorders in mice and rats. Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi) 71, 195 – 210 (1975) (text in Japanese with English abstract)
9 Hata T, Kita T, Namimatsu A, Itoh E and Oda Y: Changes of blood pressure and regional blood flow in SART rats and drug actions on these changes. Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi) 79, 335 – 342 (1982) (text in Japanese with English abstract)
10 Budd GM and Warhaft N: Body temperature, shivering, blood pressure and heart rate during a standard cold stress in Australia and Antarctica. J Physiol (Lond) 186, 216 – 232 (1966)
11 Shibahara N, Matsuda H, Umeno K, Shimada Y, Itoh T and Terasawa K: The responses of skin blood flow, mean arterial pressure and R-R interval induced by cold stimulation with cold wind and ice water. J Auton Nerv Syst 61, 109 – 115 (1996)
12 Mahboob T, Huleem DJ, Mumbaz M and Haleem MA: Stress and hypertension: role of serum, red cell and tissue electrolytes. Life Sci 58, 1587 – 1590 (1996)
13 Kita T, Hata T, Itoh E and Namimatsu A: Testing methods for vegetative syndrome in the rat and effects of Neurotropin and other drugs. Jpn J Psychosom Med 23, 61 – 68 (1983)
14 Hata T, Nishimura Y, Kita T, Kawabata A and Itoh E: Electrocardiogram in rats loaded with SART stress (repeated cold stress). Jpn J Pharmacol 45, 365 – 372 (1987)
15 Kita T, Hata T, Iida J, Yoneda R and Isida S: Decrease in pain threshold in SART stressed mice. Jpn J Pharmacol 29, 479 – 482 (1979)
16 Hata T, Kawabata A, Kita T, Itoh E and Nishimura Y: Changes in platelet count and related parameters in SART-stressed mice and the action of administered Neurotropin. Jpn J Pharmacol 47, 349 – 356 (1988)
17 Hata T, Kita T, Itoh E and Namimatsu A: Changes of the function in the heart of SART stressed (repeated cold stressed) mice and the action of Neurotropin on these changes. Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi) 79, 487 – 492 (1982) (text in Japanese with English abstract)
18 Hata T, Kita T, Kawabata A, Itoh E and Nishimura Y: Changes of tissue blood flow in mice loaded with SART (repeated cold) stress or restraint and water immersion stress and the effect of administered Neurotropin. Jpn J Pharmacol 41, 69 – 79 (1986)
19 Hata T, Kita T, Higashiguchi T and Ichida S: Total acetycholine content, and activities of choline acetyltransferase and acetylcholinesterase in brain and duodenum of SART-stressed (repeated cold-stressed) rat. Jpn J Pharmacol 41, 475 – 485 (1986)
20 Uchida S, Takeyasu K, Nomuchi Y, Yoshida H, Hata T and Kita T: Decrease in muscarinic acetylcholine receptors in the small intestine of mice subjected to repeated cold stress. Life Sci 22, 2197 – 2204 (1978)
21 Ozkutlu U, Onat F, Aslan AN and Oktay S: Central muscarinic Mcholinoreceptors involved in cholinergic hypertension. Eur J Pharmacol 250, 349 – 354 (1993)
22 Taira CA: Muscarinic receptor subtype involvement in brain cholinergic stimulation by intracerebroventricular neostigmine in sinoaortic denervated rats. Gen Pharmacol 31, 583 – 588 (1998)
23 Hata T, Kita T, Itoh E and Harada N: Experimental studies on optimal conditions of loading SART stress (repeated cold stress) upon animals. Jpn J Psychosom Med 24, 258 – 266 (1984)
24 Lawler JE, Barker GF, Hubbard JW and Schaub RG: Pathophysiological changes associated with stress-induced hypertension in the borderline hypertensive rat. Clin Sci 59, 307s – 310s (1980)
Stress-Induced Hypotension and AF-DX 116

Drolet G, Beaulieu J, Mansi JA, Champagne D and Laforest S: Relationship between exposure to stress and development of hypertension. Ann Endocrinol (Paris) 56, 187 – 191 (1995) (text in French with English abstract)

Nordin M, Morat P and Zainora M: The effect of endogenous opioids on blood pressure during stress. Clin Exp Pharmacol Physiol 14, 303 – 308 (1987)

Fisher LA and Brown MR: Central regulation of stress responses: regulation of the autonomic nervous system and visceral function by corticotropin releasing factor-41. Baillieres Clin Endocrinol Metab 5, 35 – 50 (1991)

Ruszynah BH, Nabishah BM, Aminuddin S and Khalid BA: Mineralocorticoid and glycyrrhizic acid block stress induced hypotension in rats. Clin Exp Pharmacol Physiol 22, 35 – 39 (1995)

Pavcovich LA and Valentino RJ: Regulation of a putative neurotransmitter effect of corticotropin-releasing factor: effects of adrenalectomy. J Neurosci 17, 401 – 408 (1997)

Peralta EG, Ashkenazi A, Winslow JW, Smith DH, Ramachandran J and Capon DJ: Distinct primary structures, ligand-binding properties and tissue-specific expression of four human muscarinic acetylcholine receptors. EMBO J 6, 3923 – 3929 (1987)

Hammer R, Giraldo E, Schiavi GB, Monferini E and Ladinsky H: Binding profile of a novel cardioselective muscarinic receptor antagonist, AF-DX 116, to membranes of peripheral tissues and brain in the rat. Life Sci 38, 1653 – 1662 (1986)

Aslan N, Goren Z, Ozkutlu U and Oktay S: Modulation of pressor response elicited by carbachol and electrical stimulation of the amygdala by muscarinic antagonists in conscious rats. Br J Pharmacol 121, 35 – 40 (1997)

Pelat M, Lazartigues E, Tran MA, Gharib C, Montastruc JL, Montastruc P and Rascol O: Characterization of the central muscarinic cholinoreceptors involved in the cholinergic pressor response in anesthetized dogs. Eur J Pharmacol 379, 117 – 127 (1999)

Pitschner HF, Schulte B, Schlepper M, Palm D and Wellstein A: AF-DX 116 discriminates heart from gland M2-cholinoceptors in man. Life Sci 45, 493 – 498 (1989)

Mukaiyama O, Takeuchi A, Kimura T and Satoh S: Effects of pirenzepine and AF-DX 116 on ganglionic transmission in the cardiac sympathetic nerves of the dog: interaction of M1 and M2 receptors with nicotinic receptors. J Pharmacol Exp Ther 256, 525 – 529 (1991)

Schwegler M and Jacob R: Catecholamine antagonism of acetylcholine and dibutyrylguanosine 3’,5’-monophosphate in the mammalian ventricular myocardium. Recent Adv Stud Cardiac Struct Metab 7, 391 – 399 (1975)

Loiacono RE, Rand MJ and Story DF: Interaction between the inhibitory action of acetylcholine and the alpha-adrenoceptor autoinhibitory feedback system on release of [3H]-noradrenaline from rat atria and rabbit ear artery. Br J Pharmacol 84, 697 – 705 (1985)

Miyazoe H, Harada Y, Yamasaki S and Tsuji Y: Clinical study on accentuated antagonism in the regulation of heart rate in children. Jpn Heart J 39, 481 – 487 (1998)

McPhail LT and Jones DR: The autonomic nervous control of heart rate in ducks during voluntary diving. Physiol Biochem Zool 72, 164 – 169 (1999)

Muscholl E: Peripheral muscarinic control of norepinephrine release in the cardiovascular system. Am J Physiol 239, H713 – H720 (1980)

Baskin SI and Thomsen RH: The effect of AF-DX 16, a cardioselective muscarinic antagonist, on the negative inotropic action of acetylcholine. Res Commun Chem Pathol Pharmacol 71, 3 – 16 (1991)