Pharmacological Analysis of Cardiac Effects of a Central Cholinergic Agent, WEB 1881-FU, in Isolated Canine Heart Preparations

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SUMMARY

The cardiovascular effects of the novel compound WEB 1881-FU (4-amino-methyl-1-benzyl-pyrrolidine-2-one-fumarate) were investigated using cross-circulated dog atrial preparations. Intravenous injections of WEB 1881 induced a hypotensive effect with slight tachycardia at a large dose in anesthetized dogs. At the same time, it produced slight positive chronotropic and inotropic effects in the isolated atrium perfused with the donor's arterial blood. Direct injections of WEB 1881 into the sinus node artery of the isolated and perfused atrium induced positive chronotropic and inotropic effects in a relatively small dose range. At higher doses, it caused biphasic chrono- and inotropic effects, i.e., initial brief negative effects followed by long-lasting positive chrono- and inotropic effects. The negative chrono- and inotropic responses to WEB 1881 were not blocked by atropine in doses which significantly suppressed ACh-induced negative chrono- and inotropic responses. The positive chrono- and inotropic responses to WEB 1881 were significantly inhibited either by a beta-blocker (propranolol) or by an uptake blocker (imipramine). From these results, it is concluded that WEB 1881 has a tyramine-like, catecholamine releasing property. Although a large dose of WEB 1881 has a direct cardiac depressant action, it does not affect muscarinic mechanisms in the heart.

Additional Indexing Words:

Isolated dog atrium Cross-perfused atrial preparation WEB 1881-FU

A variety of drugs for treatment of brain dysfunction in the elderly are being widely investigated.^{1),2)} WEB 1881-FU (4-amino-methyl-1-benzyl-pyrrolidine-2-one-fumarate) is a central cholinergic agent which enhances cognition and cerebral metabolism, and is a potential agent for treatment of impaired memory and cognition in the elderly.³⁾ However, the SA node area is densely innervated by cholinergic nervous elements and the SA nodal pacemaker activity is very sensitive to cholinergic substances. Thus,

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we investigated the cardiovascular effects of this novel potent central cholinergic agent, WEB 1881-FU, using cross-circulated dog atrial preparations.^{4),5)}

METHODS

Thirty-eight mongrel dogs of either sex, weighing 8 to 20 kg, were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Immediately after intravenous administration of sodium heparin (500 units/kg), the right atrium was excised and plunged into cold physiological saline at 4 to 10°C. The right atrium was isolated and cross-perfused with heparinized arterial blood from a donor dog according to the procedure described previously.^{4),5)}

Briefly, the sinus node artery of the isolated right atrium was cannulated with polyethylene tubing at its origin in the right coronary artery. All arterial branches, except the sinus node artery, were carefully ligated. The excised right atrium was placed in a glass chamber and perfused with arterial blood from the carotid artery of the donor dog using a peristaltic pump (Harvard Apparatus, model 1210). The perfusion pressure was kept constant at 100 mmHg. The perfusion flow rate at this pressure was approximately 2 to 8 ml/min and the time delay from the donor dog to the atrium was 2 to 4 min. The glass chamber was filled with blood and maintained at a constant temperature of 37°C. Bipolar platinum electrodes were placed in contact with the atrial epicardium. The sinus rate was measured with a tachometer triggered by atrial electrograms, and isometric tension development was measured with a force displacement transducer (Grass FTO3B). The atrial muscle was subjected to a resting tension of 2 g. The donor dogs were respired artificially with room air using a respirator (Harvard Apparatus, model 607). The systemic blood pressure and heart rate in the donor dog and the sinus rate and developed tension in the isolated atrium from another dog were recorded simultaneously on a polygraph (Nihon Kohden). Drugs used were as follows: WEB 1881-FU (WEB 1881) (4-amino-methyl-1-benzyl-pyrrolidine-2-one-fumarate, Boehringer Ingelheim), norepinephrine hydrochloride (Sankyo), tyramine hydrochloride (Tokyo Kasei), imipramine hydrochloride (Fujisawa), acetylcholine chloride (ACh, Daiichi), atropine sulfate (Takeda) and propranolol hydrochloride (Sumitomo).

The drug solution was either injected intravenously in a volume of 0.1–1 ml over a period of 10 sec into the jugular vein of the donor dog, or administered in a volume of 10 to 30 μ l over a period of 4 sec into the rubber tubing which conducted the blood to the arterial cannula of the isolated atrium preparation. The data were analyzed by analysis of variance for

multiple comparisons of data and Student's t-test for paired data was assessed for comparison between 2 groups. P values of less than 0.05 were considered significant.

RESULTS

Effects of intravenous administration of WEB 1881 to the anesthetized donor dog which was cross-circulated to the isolated atrium:

When WEB 1881 was administered intravenously to the donor dog, a depressor response was produced dose-dependently with slight increases in heart rate at 1–100 $\mu\text{mol/kg}$. At a large dose (greater than 10 $\mu\text{mol/kg}$ i.v.), WEB 1881 induced slight increases in atrial rate and developed tension of the isolated atrium. Figure 1 shows typical responses to intravenous administration of WEB 1881 to a donor dog. The chrono- and inotropic responses of the isolated atrium began approximately 3 min after the injection, because intravenous WEB 1881 requires several min to reach the isolated atrium. The data are summarized in Fig. 2.

Effects of intra-arterial injection of WEB 1881 into the cannulated sinus node artery of the isolated atrium:

When norepinephrine was administered into the sinus node artery of the isolated atrium, positive chrono- and inotropic responses were produced in a dose-related manner. Intra-arterial injection of WEB 1881 induced

Fig. 1. Cardiovascular effects of WEB 1881 (100 $\mu\text{mol/kg}$), injected intravenously to a donor dog. (A) Blood pressure and heart rate traces from a donor dog, anesthetized with sodium pentobarbital and respired with room air. (B) Sinus rate and developed tension traces from an isolated atrial preparation, perfused with the donor's arterial blood.

Fig. 2. Cardiovascular effects of intravenous injections of WEB 1881 in the donor dog. The control values of systemic blood pressure (SBP) of donor dogs and heart rate (HR) were 95 ± 28 mmHg (mean $\pm$ SEM) and 164 ± 20 beats/min, respectively, in 3 donor dogs. The control atrial rate (AR) and the developed tension (T) were 100 ± 3 beats/min and 3.4 ± 0.4 g, respectively, in 3 isolated atria.

slight positive chrono- and inotropic effects at doses of 100 nmol to 30 μmol . Increasing doses of WEB 1881 (over 3 μmol) usually caused biphasic chrono- and inotropic effects; brief negative effects were followed by long-lasting positive chrono- and inotropic effects. Figure 3 shows a typical dose-dependent chrono- and inotropic effect of increasing doses of WEB 1881. The cardiac responses to WEB 1881 were reproducible (Fig. 4). The maximum increase in sinus rate by WEB 1881 was approximately 10%, while the maximum increase in developed tension was approximately 20%. By contrast, 1 nmol norepinephrine produced increases of 40% and 120%, respectively. Data are summarized in Fig. 5.

Effects of atropine on WEB 1881- or ACh-induced cardiac responses:

Negative chrono- and inotropic responses were induced in a dose-related

Fig. 3. Effects of injections of WEB 1881 into the sinus node artery of the isolated atrial preparation. Dose dependent chronotropic and inotropic effects were observed.

Fig. 4. Chronotropic and inotropic responses to repetitive injections of 10 μmol WEB 1881 into the sinus node artery of the isolated atrium.

Fig. 5. Effects of WEB 1881 on norepinephrine (NE)- and WEB-induced chronotropic and inotropic responses in the isolated dog atria. The control atrial rate was 102 ± 8 beats/min (n=5 atria); the control developed tension was 3.8 ± 0.4 g (n=5 atria).

manner when ACh was injected into the cannulated sinus node artery. The ACh-induced negative cardiac effects were readily inhibited by atropine treatment. The WEB 1881-induced negative chrono- and inotropic responses were not inhibited by atropine at doses which completely inhibited the ACh-induced negative cardiac responses. Data are summarized in Fig. 6.

Effects of propranolol on WEB 1881- or norepinephrine-induced positive chrono- and inotropic responses:

Propranolol produced negative chronotropic and inotropic effects in the isolated atrium when injected into the sinus node artery. Both WEB 1881- and norepinephrine-induced positive chrono- and inotropic responses were significantly inhibited by treatment with propranolol (Fig. 7).

Effects of imipramine on WEB 1881- and tyramine-induced positive chrono- and inotropic responses:

Injections of tyramine into the sinus node artery produced positive chrono- and inotropic responses in a dose-related manner. As reported previously,^{7),8)} these tyramine-induced positive cardiac responses were inhibited by treatment with either beta-blockade or uptake blockade. The positive chrono- and inotropic responses to WEB 1881 were also blocked by treatment with a potent uptake blocker, imipramine. Figure 8 illustrates the blocking effects of imipramine on tyramine- and WEB 1881-induced positive cardiac responses.

Fig. 6. Effects of 1 nmol atropine on negative chronotropic and inotropic responses to 0.1 nmol acetylcholine (ACh) and 30 μ mol WEB 1881. The control atrial rate and developed tension were 109 ± 8 beats/min and 3.6 ± 0.2 g, respectively ($n = 5$ atria).

Fig. 7. Effects of 10 nmol propranolol on positive chronotropic and inotropic effects of 0.3 nmol norepinephrine (NE) and 30 μ mol WEB 1881 (n=5 isolated dog atria).

Fig. 8. Effects of 100 nmol imipramine on chronotropic and inotropic responses to 10 nmol tyramine (TYR) and 30 μ mol WEB 1881 (n=5 isolated dog atria).

DISCUSSION

WEB 1881 is a recently developed compound with central cholinomimetic and cytoprotective properties which has also been reported to enhance metabolism and cognitive performance.³⁾ Kuhn et al³⁾ reported that WEB 1881 slightly decreased heart rate and blood pressure of spontaneously hypertensive rats, although not significantly at 10 and 30 mg/kg, p.o. There

are no available reports concerning cardiovascular responses to WEB 1881 in dogs.

In the present study, we demonstrated that intravenous injection of WEB 1881 induced a slight hypotension and tachycardia in anesthetized dogs. Moreover, we confirmed that WEB 1881 injected into the sinus node artery produced positive chrono- and inotropic effects. The significant attenuation of WEB 1881-induced positive cardiac effects by beta-blockade suggests that adrenergic mechanisms are involved in these effects. Both the WEB 1881-induced positive chrono- and inotropic effects and the tyramine-induced effects were inhibited by treatment with a potent uptake blocker, imipramine. These findings indicate that WEB 1881-induced positive cardiac actions may reflect a release of catecholamines from sympathetic nerve terminals in the SA node area.⁶⁾ Since nicotine-induced positive chronotropic effects are inhibited by tetrodotoxin,^{7),8)} the WEB-induced catecholamine release is likely to differ from a nicotine-like action. In 1979, Kobayashi et al⁹⁾ demonstrated that acetaldehyde can evoke a release catecholamine at the sympathetic nerve terminals in the dog SA node area. They reported that acetaldehyde-induced positive chrono- and inotropic effects are potentiated by imipramine treatment. These findings were reproduced in isolated blood vessel preparations.^{10),11)} Thus, the WEB 1881-induced catecholamine release may indicate a tyramine-like action, rather than a nicotine- or an acetaldehyde-like action.

At large doses, WEB 1881 often induced initial, brief negative chrono- and inotropic effects. These negative cardiac effects were not modified by atropine in doses which significantly suppressed ACh-induced negative cardiac actions. Therefore, these negative cardiac actions probably reflect direct cardiac depressant properties.

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