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O-Acetyljervine: A New β-adrenoceptor Agonist from *Veratrum album*

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Intravenous administration of *O*-acetyljervine (an alkaloid from Vertrum album) produced a dose-dependent (10-100 μg/kg) fall in blood pressure and tachycardia in anaesthetized normotensive rats. Pretreatment of animals with propranolol (1 mg/kg) abolished these cardiovascular responses of *O*-acetyljervine similar to that of isoprenaline (1 μ/kg). In isolated tissue experiments, *O*-acetyljervine (10-100 μ/ml) produced a dose-dependent relaxation of phenylephrine-induced contraction of the rabbit aorta. In guinea-pig spontaneously beating atria, it caused positive inotropic and chronotropic responses in a dose-dependent fashion (10-100 μ/ml). These responses were abolished in the presence of propranolol (1 μg/ml) similar to that of isoprenaline. These results indicate that *O*-acetyljervine is a adrenoceptor stimulant (β_1 and β_2) like isoprenaline.

Key words : *O*-acetyljervine, *Veratrum album*, β-adrenoceptor agonist, Hypotensive, Cardiac stimulant

INTRODUCTION

Veratrum species (Melanithiaceae) are well known for their hypotensive constituents and we have recently isolated three new hypotensive alkaloids (jervinone, *O*-acetyljervine and 1-hydroxy-5,6-dihydrojervine) from the rhizomes of *Veratrum album* (Atta-ur-Rahman et al., 1992, 1993).

The hypotensive action of jervinone was blocked by atropine similar to that of acetylcholine whereas *O*-acetyljervine and 1-hydroxy-5,6-dihydrojervine were non-cholinergic in action as the effect was not modified by the pretreatment of animals with atropine (Atta-ur-Rahman *et al.*, 1993). *O*-acetyljervine was available in a small quantity and studied further for its possible mechanism of hypotensive action.

MATERIALS AND METHODS

Drugs

Isoprenaline hydrochloride, phenylephrine hydrochloride and propranolol hydrochloride were obtained from Sigma Chem. Co. St. Louis, MO, USA and pentothal sodium was obtained from Abbott Laboratories Pakistan. *O*-acetyljervine was isolated from *Vertrum album* in our laboratory (Atta-ur-Rahman, et al., 1992). Stock solutions of all drugs including *O*-acetyljervine were prepared in distilled water and dilutions were made in normal saline (0.9% NaCl).

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Anaesthetized rats

In these experiments, adult male Sprague-Dawley rats (200-250 g) were used and arterial blood pressure was recorded as described previously (Gilani, 1991). The animals were anaesthetized with an intraperitoneal injection of sodium thiopentone (Pentothal, 50-70 mg/kg body weight). The right carotid artery was cannulated with heparinized polyethylene tubing PE-50, which was connected to a pressure transducer (Statham P23 AC) coupled with a (Grass model 79 D) polygraph. Heart rate was measured from the lead II ECG by using a Grass tachograph (model 7 PAF).

The left jugular vein was cannulated with similar tubing to facilitate the intravenous injection of the drugs. The exposed surface with the cannulation was covered with cotton wool moistened in warm saline. The rats were injected with heparin (1000 units/kg body weight) to prevent blood clotting.

After a 20 minute period of equilibrium, the rats

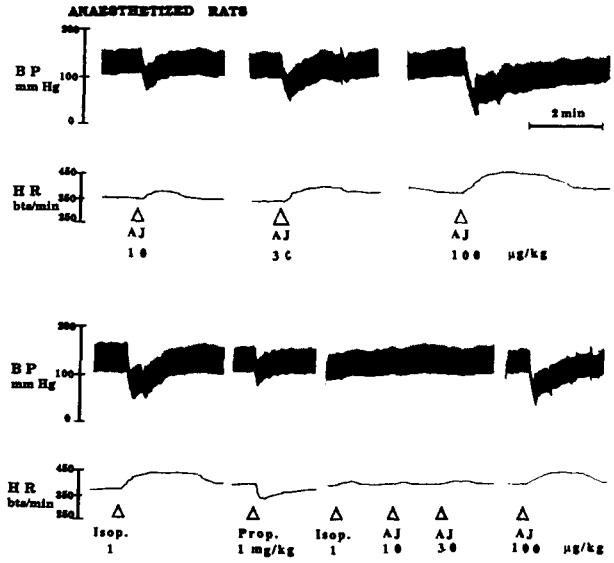

Fig. 1. A representative tracing showing comparison of *O*-acetyljervine (AJ) and isoprealine (Isop) for their effects on blood pressure (BP) and heart rate (HR) in the absence and presence of prapranolol (Prop) in anaesthetized rats. Propranolol was administered 5 minutes before the redetermination of AJ or Isop. responses.

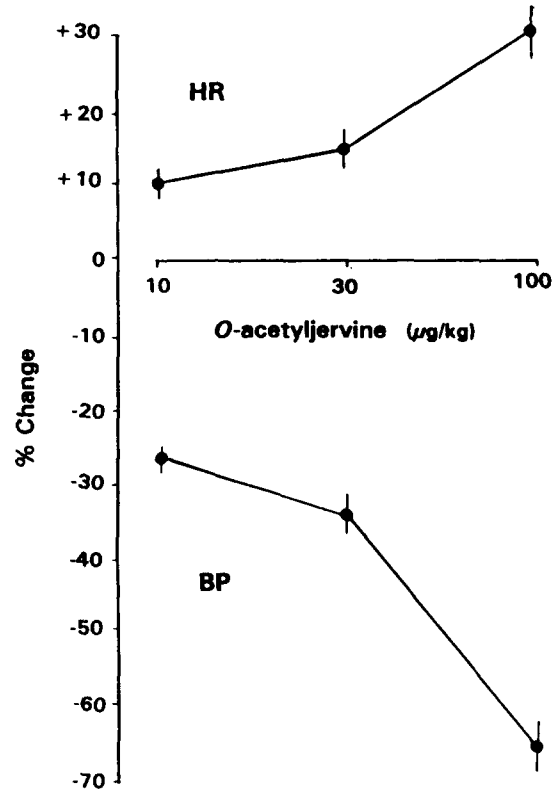

Fig. 2. Dose-dependent effects of *O*-acetyljervine (n=5) on blood pressure (BP) and heart rate (HR) in anaesthetized rats

were injected intravenously with 0.2 ml saline or with the same volume of test substance(s). Arterial blood pressure was allowed to return to the resting level between injections. Changes in blood pressure and heart rate were recognized as the difference between the steady state values before and the peak readings after injection. Mean blood pressure was calculated as the diastolic blood pressure plus one-third pulse width.

Isolated tissue experiments

These experiments were carried out by the method previously described (Gilani, 1989; 1991).

Guinea-pig atria

Guinea-pigs of either sex (400-600 g) were killed by cervical dislocation. Paired atria were removed carefully and mounted into a 20 ml tissue bath filled with Krebs-Henseleit solution maintained at 35°C and aerated with 5% carbon dioxide in oxygen. The composition of the physiological salt solution was (mM): NaCl, 118.2; KCl, 4.7; $MgSO_4$, 1.3; KH2PO4, 1.2; D-glucose, 11.7; $NaHCO_3$, 25.0 and $CaCl_2$, 2.5. The spontaneous atrial contractions were recorded via a force-displacement transducer (FT-03) together with rate of contractions which were monitored with a Grass model 7 PAF tachograph. All experimental records were made on a Grass model 79 D polygraph. The preparation was allowed to equilibrate under 1 g resting tension for at least 30 min before administration of any drug.

Rabbit thoracic aorta

New Zealand white rabbits of either sex weighing 2-3 kg were killed by a blow to the back of the head. The descending thoracic aorta was quickly removed and cut into rings of 2-3 mm width which were opened by cutting perpendicular to the axis of symmetry of the cylindrical vessel to make strips. Each strip preparation was mounted in a 20 ml tissue bath containing Krebs-Henseleit solution, maintained at 37°C and continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide. A resting tension of 2 g was applied to each tissue and an equilibrium period of one hour was allowed before changes in isometric tension of the strips were measured as described for atria.

Drugs were added to the tissue bath in a cumulative fashion (Van Rossum, 1963) and the concentrations mentioned in the text or figures represent final bath concentrations.

RESULTS

Anaesthetized rats

Intravenous administration of *O*-acetyljervine (AJ) caused a fall in mean arterial pressure (27-66%) and a rise in heart rate (10-32%). These cardiovascular actions were dose-dependent mediated at the dose range of 10-100 µg/kg. Fig. 1 shows the representative tracing from a typical experiment whereas the combined effects of different experiments are presented in Fig. 2. Isoprenaline (1 µg/kg) also caused changes in blood pressure and heart rate qualitatively similar to that caused by *O*-acetyljervine. Pretreatment of rats with propranolol (1 µg/kg) completely abolished the cardiovascular effects of isoprenaline (1 µg/kg) and *O*-acetyljervine up to the dose of 30 µg/kg. However, the effect of higher doses of AJ (100 µg/kg) was blocked partially (Fig. 1).

Isolated Rabbit Aorta

Phenylephrine (0.1 µg/ml) caused a sustained contraction which allowed for the collection of dose-response (relaxation) data. *O*-acetyljervine (10-100µg/ml) caused a dose-dependent relaxation of phenylephrine-induced contraction of rabbit aorta (Fig 3) similar to that caused by isoprenaline (0.1-1 µg/ml). The tissue was pretreated with propranolol (1 µg/ml) to block β-adrenoceptors. In the presence of propranolol, both isoprenaline and O-acetyljervine failed to cause relaxation of phenylephrine-induced contractions.

Isolated guinea-pig atria

O-acetyljervine (10-100 µg/ml) caused a dose-dependent increase in force (up to 86%) and rate (up to 59%) of atrial contractions. Isoprenaline was also included in the study for comparison which caused a pronounced increase in force and rate of atrial contractions at 0.1 µg/ml. However, when these agents were repeated in the presence of propranolol (1 µg/ml), they failed to produce any change in force or rate of spontaneous atrial contractions.

DISCUSSION

O-acetyljervine produced a fall in blood pressure and caused tachycardia in anaesthetized normotensive rats. Pretreatment of animals with propranolol abolished the cardiovascular responses to AJ as well as to isoprenaline, although the hypotensive and tachycardiac effects of higher doses of AJ was only partially blocked. Isoprenaline is known to produce a fall in blood pressure by activation of β_2-adrenoceptors in

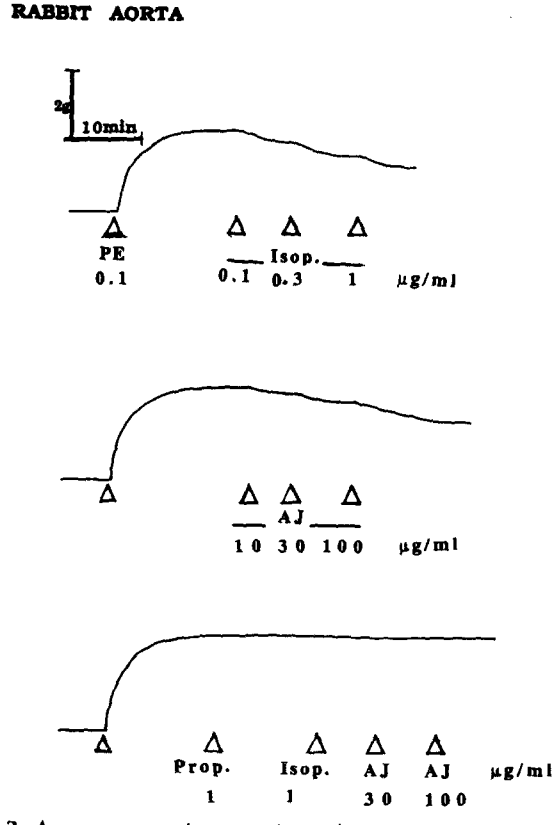

Fig. 3. A representative tracing showing comparison of isoprenaline (Isop.) and *O*-acetyljervine (AJ) in the absence and presence of propranolol (Prop) on phenylephrine (PE)-induced contractions of isolated rabbit aorta

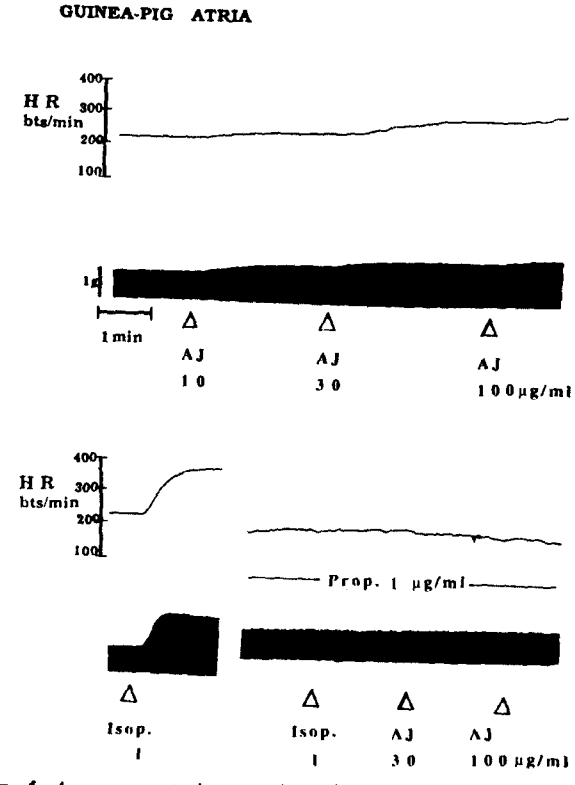

Fig. 4. A representative tracing showing comparison of *O*-acetyljervine (AJ) and isoprenaline (Isop.) on force and rate of atrial contractions in the absence and presence of propranolol (Prop)

the blood vessels and tachycardia by activation of β_1-adrenceptors in the heart (Hoffman and Lefkowitz, 1990a). Propranolol is a competitive antagonist of isoprenaline at both β_1 & β_2 adrenoceptors (Hoffman and Lefkowitz, 1990b). Therefore, blockade of *O*-acetyljervine responses by propranolol suggests that the hypotensive and tachycardiac responses of this drug are mediated through mechanisms similar to that of isoprenaline. The fact that the high dose of AJ produced a partial response in the propranolol treated animals suggests that AJ displaced some of the propranolol from receptors as a result of competition between agonist and antagonist. It is a characteristic of pharmacological agonists (receptors activating drugs) that increasing doses can restore the response by displacing antagonist from receptors (Arunlakshana and Schild, 1959).

The β-adrenoceptor stimulant activity of *O*-acetyljervine was confirmed when it was tested on isolated tissue preparations, such as, rabbit aorta and guinea-pig paired atria. In aorta, AJ produced relaxant effect which was blocked by propranolol similar to that of isoprenaline. Similarly, AJ also produced an increase in rate and force of atrial contractions similar to that of isoprenaline as responses to both of these agents were blocked by propranolol. Propranolol is also known to exhibit membrane stablising action at high doses, but the inhibitory effect of propranolol against AJ or isoprenaline in this study cannot be attributed to this action as the dosages used were distinctly lower than required for direct effect on membranes (Hoffman and Lefkowitz, 1990b).

Thus, it is clear from these results that the pure compound *O*-acetyljervine isolated from rhizomes of *Veratrum album* exhibits isoprenaline like actions (β_1 and β_2 and adrenoceptor stimulant) and the mechanism of its hypotensive action is different from the other compound (jervinone) isolated from the same plant (Atta-ur-Rahman *et al.*, 1993).

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