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Short communication

U50,488H-induced pressor effect in the ovine foetus is mediated by sympathetic activation and vasopressin

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Abstract

The purpose of this study was to investigate the mechanisms behind the increase in blood pressure observed after intravenous administration of U50,488H (*trans*-3,4-dichloro-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide), a selective κ-opioid receptor agonist, to the ovine foetus. Intravenous administration of U50,488H (1.0 mg/kg) to the foetus resulted in an immediate increase in foetal blood pressure which lasted 15 min. Pretreatment with phentolamine (1.0 mg/kg i.v.) completely blocked the immediate (1–4 min) pressor effect of U50,488H, but not the subsequent increase in blood pressure after 5 min. In contrast, pretreatment with the vasopressin antagonist ([β-mercapto-β,β-cyclopentamethylene-propionyl)-*O*-Me^2-Tyr,Arg^8]vasopressin, 0.06 mg/kg) did not affect the immediate pressor effect of U50,488H, but completely blocked the latter increase in blood pressure after 4 min. These data suggest that the immediate increase in blood pressure caused by U50,488H was mediated by sympathetic activation which was then further sustained by a release of vasopressin.

Keywords: κ-Opioid receptor; Cardiovascular; Blood pressure; Phentolamine; Pregnancy

1. Introduction

We recently reported that intravenous (i.v.) administration of U50,488H (*trans*-3,4-dichloro-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide), a selective κ-opioid receptor agonist, to the ovine foetus resulted in a significant increase in arterial blood pressure and heart rate which lasted approximately 15 min (Szeto et al., 1996). This was in contrast to most findings in the adult, where i.v. U50,488H has been reported to reduce arterial blood pressure and heart rate in the rat (Pugsley et al., 1992; Hall et al., 1988). We had evidence that this effect of U50,488H on the foetal cardiovascular system was mediated by opioid receptors, although the specific mechanisms remained unclear (Szeto et al., 1996). In this paper, we present evidence that the immediate elevation in blood pressure after U50,488H is due to activation of the sympathetic nervous system. However, this κ-opioid receptor-mediated sympathetic activation is very short-lived, and the more sustained pressor effect is due to a release of vasopressin.

2. Materials and methods

2.1. Animal preparation

These studies were carried out in 18 unanaesthetized foetal sheep with gestational ages ranging from 124 to 141 days (term being ~ 145 days). 5 or more days prior to the study, chronic indwelling catheters were implanted in the foetal sheep in accordance with guidelines approved by the Institution for the Care and Use of Animals at Cornell University Medical College. Details of the surgical procedures have been described previously (Szeto, 1983; Szeto et al., 1996). Briefly, a polyvinyl catheter was placed in the foetal aorta for continuous recording of foetal blood pressure and blood sampling. Two catheters were placed in the foetal inferior vena cava, one for the administration of U50,488H and the other for the administration of the antagonists.

2.2. Drug design

Foetal blood pressure was monitored continuously for at least 2 h before and 3 h after drug administration. Animals were included in the study only if foetal arterial blood

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gases were within the normal range (pO_2 = 16–25 mm Hg, pCO_2 = 40–55 mm Hg, and pH = 7.33–7.40). In 8 animals, U50,488H (RBI, Natick, MA, USA; 1.0 mg/kg estimated foetal weight (Szeto et al., 1996)) was administered to the foetus i.v. over 1.0 min. In other animals, the same dose of U50,488H was administered 10 min after either the administration of phentolamine (Ciba, Summit, NJ, USA; 1.0 mg/kg i.v., $n = 5$) or [β-mercapto-β,β-cyclopentamethylene-propionyl)-*O*-Me^2-Tyr,Arg^8]vasopressin (Sigma, St. Louis, MO, USA; 0.06 mg/kg i.v., $n = 5$).

2.3. Data analysis

All data are presented as mean ± S.E.M. One-way analysis of variance (ANOVA) with repeated measure (factor = time) was used to examine the effects of U50,488H on foetal blood pressure. In cases where test of normality failed, ANOVA by ranks was used. Dunnett's test was used for post-hoc comparison of each time point to the pre-drug control. The ability of phentolamine or the vasopressin receptor antagonist to modify the responses to U50,488H was evaluated using Friedman's two-way ANOVA with repeated measure (factors = treatment, time) and post-hoc comparison at each time point using the Student-Newman-Keuls test.

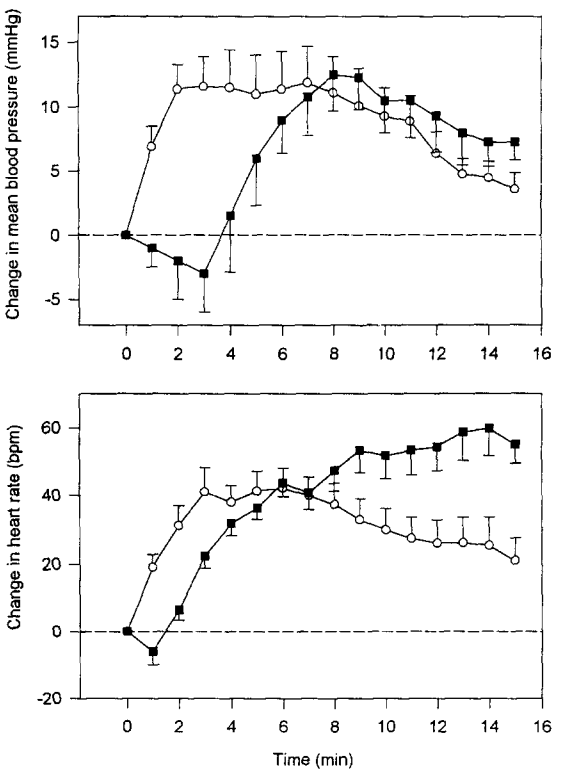

Fig. 1. (Top panel) Effects of U50,488H (1.0 mg/kg i.v.) on foetal mean blood pressure in the absence (○) ($n = 8$) and presence (■) ($n = 5$) of phentolamine (1.0 mg/kg i.v.). (Bottom panel) Effects of U50,488H on foetal heart rate in the absence (○) ($n = 8$) and presence (■) ($n = 3$) of phentolamine. Data are presented as means ± S.E. * $P < 0.05$ when compared to the same time point after U50,488H alone.

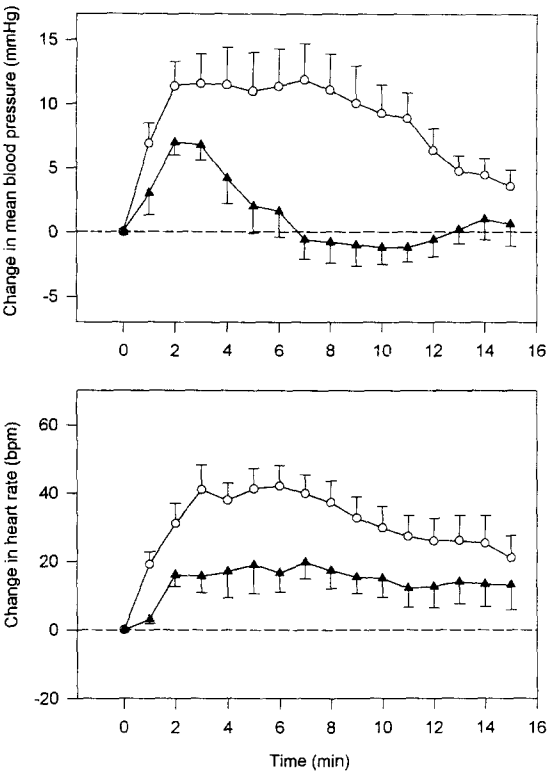

Fig. 2. (Top panel) Effects of U50,488H (1.0 mg/kg i.v.) on foetal mean blood pressure in the absence (○) ($n = 8$) and presence (▲) ($n = 5$) of the vasopressin receptor antagonist (0.06 mg/kg i.v.). (Bottom panel) Effects of U50,488H on foetal heart rate in the absence (○) ($n = 8$) and presence (▲) ($n = 5$) of the vasopressin receptor antagonist. Data are presented as means ± S.E. * $P < 0.05$ when compared to the same time point after U50,488H alone.

3. Results

Foetal mean blood pressure and heart rate prior to U50,488H administration were 42.1 ± 1.9 mm Hg and 153.6 ± 4.8 bpm, respectively. The effect of U50,488H on foetal blood pressure was highly significant ($F = 7.854$; $P < 0.001$), and post-hoc comparison revealed that mean blood pressure was significantly elevated compared to pre-drug values from 1 to 15 min ($P < 0.05$) (Fig. 1). Foetal heart rate was also significantly increased ($\chi^2 = 40.6$; $P < 0.001$) over the same time period, reaching a peak of 195.8 ± 8.6 bpm at 6 min after U50,488H administration.

Administration of phentolamine alone resulted in a transient (1–2 min) decrease in foetal mean blood pressure (5–8 mm Hg) and a significant and prolonged increase in foetal heart rate. Foetal heart rate increased by as much as 100–120 bpm and was still significantly elevated in 2 animals by the time of U50,488H administration. Foetal blood pressure just prior to U50,488H administration was

47.8 ± 3.5 mm Hg. Pretreatment with phentolamine abolished the immediate increase in foetal blood pressure following U50,488H administration, but a significant increase was still seen from 6–12 min (Fig. 1). Two-way ANOVA confirmed that phentolamine pretreatment significantly altered the blood pressure response to U50,488H ($F = 9.039$; $P = 0.019$), and post-hoc comparison revealed significant differences at 1, 2, 3 and 4 min ($P < 0.05$). In the 3 animals where foetal heart rate was near-normal when U50,488H was administered, the foetal tachycardia to U50,488H was more prolonged in the presence of phentolamine.

Administration of the vasopressin receptor antagonist had no significant effect on either foetal blood pressure or heart rate. Foetal blood pressure and heart rate prior to U50,488H administration were 46.6 ± 1.5 mm Hg and 163.4 ± 4.4 bpm, respectively. Pretreatment with the vasopressin receptor antagonist did not affect the immediate pressor response to U50,488H, but the duration of the response was greatly shortened (Fig. 2). The blood pressure response to U50,488H in the presence of the vasopressin receptor antagonist was significantly different from U50,488H alone ($F = 18.273$; $P = 0.004$), and post-hoc comparison revealed significant differences from 5–9 min ($P < 0.05$). Pretreatment with the vasopressin receptor antagonist attenuated the foetal heart rate response to U50,488H ($F = 4.946$; $P = 0.042$), but the increase in foetal heart rate was still statistically significant ($F = 2.105$; $P = 0.019$).

4. Discussion

The results of this study confirm our previous report that systemic administration of U50,488H results in a rapid and significant increase in foetal blood pressure (Szeto et al., 1996). In contrast, most studies in the adult have reported a decrease in blood pressure and heart rate following i.v. administration of U50,488H and other selective κ-opioid receptor agonists (Gulati and Bhargava, 1988; Hall et al., 1988; Pugsley et al., 1992). It is unclear why the effects in the ovine foetus are so different from the adult, but part of the discrepancy may be due to the presence of anesthetic agents in the adult studies. Hall et al. (1988) reported that when cats were anaesthetized with chloralose, which produces less depression of sympathetic tone than pentobarbital, spiralodine did not lower blood pressure. Furthermore, i.v. administration of bremazocine to unanaesthetized rats actually increased blood pressure in a dose-dependent manner (Salas et al., 1989). The cardiovascular actions of U50,488H also appear to be dependent on the site of administration as direct administration of U50,488H into the lateral ventricle (May et al., 1989), nucleus ambiguus (Samuels and McIntosh, 1993) or hypothalamic paraventricular nuclei (Bachelard and Pitre, 1995) were all found to have no effect on blood pressure in the anaesthetized rat. Bremazocine also had no effect on blood pressure when administered into the lateral ventricle (Salas et al., 1989). However, Carter and Lightman (1985) reported a pressor effect when U50,488H was administered directly into the nucleus tractus solitarius.

These cardiovascular actions of U50,488H in the ovine foetus were previously shown to be mediated by opioid receptors as they were completely blocked by naloxone pretreatment (Szeto et al., 1996). The purpose of this study was to further investigate the mechanisms of action of U50,488H on blood pressure regulation. Our data show that the immediate rise in blood pressure was blocked by pretreatment with phentolamine, suggesting involvement of the sympathetic nervous system. The dose of phentolamine was chosen based on previous reports of its use in α-adrenergic receptor blockade in the ovine foetus (Harris and Van Petten, 1978; Jones and Ritchie, 1978; Giussani et al., 1993). The ovine foetal circulation is reactive to α-adrenergic receptor agonists by mid-gestation although its sensitivity is significantly less than in the newborn or adult even in late gestation (Woods et al., 1977; Harris and Van Petten, 1978). This dampened response to the vasoconstrictive action of α-adrenergic receptor agonists in the foetus is thought to be due to the presence of the low-resistance umbilical-placental circulation and other vascular shunts.

A significant pressor effect was reported when U50,488H was administered unilaterally into the nucleus tractus solitarius in conscious adult rats (Carter and Lightman, 1985). This pressor effect to U50,488H was, however, not antagonized by phenoxybenzamine, leading the authors to conclude that it was not due to sympathetic activation. In the present study, the immediate elevation of blood pressure after i.v. U50,488H administration may be the result of increased norepinephrine release in the local vascular bed rather than at the central brain sites. U50,488H has been reported to increase renal sympathetic nerve activity in conscious rats (Kapusta and Obih, 1993). Interestingly, U-62066E (another κ-opioid receptor agonist) was reported to increase plasma norepinephrine in man, although it had no effect on either blood pressure or heart rate (Rimoy et al., 1994). This study, however, only used a single dose of U-62066E and higher doses may alter blood pressure and heart rate.

Although sympathetic activation may account for the rapid rise in foetal blood pressure, its action is short-lived due to the rapid degradation of catecholamines in vivo. The results of the present study suggest that this pressor effect to U50,488H is sustained by the subsequent release of vasopressin, which is known to be a potent vasoconstrictor (Cowley et al., 1974). Direct infusion of vasopressin to the ovine foetus has been reported to produce an increase in foetal blood pressure and decrease in heart rate (Rurak, 1978; Iwamoto et al., 1979; Wiriyathian et al., 1983; Tomita et al., 1985). Furthermore, in support of a role for vasopressin in this pressor response, we have

found that this dose of U50,488H produced a significant release of adrenocorticotropin which was attenuated by the same dose of vasopressin receptor antagonist, suggesting that U50,488H must have caused a release of vasopressin which then acted as an adrenocorticotropin secretagogue in the anterior pituitary (Taylor et al., 1996). These data are consistent with the report by Carter and Lightman (1985) who found that the pressor response to U50,488H, when administered to the nucleus tractus solitarius in the adult rat, was associated with an increase in plasma vasopressin levels, and that the pressor response was not observed in vasopressin-deficient animals.

The increase in foetal heart rate following U50,488H in the presence of a significant elevation of arterial pressure suggests direct sympathetic activation of the heart. The baroreflex is present by this stage of development in the ovine foetus, although it may be less effective than in the adult (Harris and Van Petten, 1978; Oakes et al., 1980; Cheung and Brace, 1987). The pressor response to exogenously administered α-adrenergic receptor agonists is usually accompanied by a decrease in heart rate as a result of baroreceptor activation. This initial bradycardia is followed by an increase in heart rate which is thought to be due to sympathetic activation of the heart. The enhanced tachycardia to U50,488H in the presence of phentolamine lends support for direct sympathetic activation of the heart by U50,488H. Blockade of α_2-adrenergic receptors by phentolamine increases sympathetic outflow and potentiates the release of norepinephrine from nerve endings. Furthermore, the lack of an initial bradycardia suggests that the baroreflex is blunted by U50,588H. Several opioid agonists have been shown to attenuate baroreflex sensitivity (Hassen and Feuerstein, 1987; Yukimura et al., 1981; Gordon, 1986), and the site of action is thought to be in the nucleus tractus solitarius (Gordon, 1990). Thus, the foetal tachycardia in response to U50,488H is probably due to sympathetic activation and blunting of the baroreflex.

In the presence of the vasopressin receptor antagonist, the magnitude of heart rate response to U50,488H was significantly attenuated. The reason behind this is not clear as vasopressin is not thought to have any direct actions on the heart. It is possible that vasopressin may potentiate the action of the sympathetic nervous system on the heart. Vasopressin has been reported to augment vasoconstriction in response to sympathetic stimulation in certain local vascular beds (Schmid et al., 1982).

It should be noted, however, that a number of investigators have reported a dose-dependent inhibition of vasopressin release by U50,488H and other κ-opioid receptor agonists which accounts for their diuretic action (Oiso et al., 1988; Leander, 1983; Huidobro-Toro and Parada, 1985). This apparent contradiction in the action of U50,488H on vasopressin is not understood but may be explained by different sites of action within the central nervous system. The suppression of vasopressin is thought to be mediated by activation of κ-opioid receptors in the posterior pituitary, while activation of κ-opioid receptors in the nucleus tractus solitarius may result in vasopressin release. It is unfortunate that blood pressure data were not available from the studies of U50,488H on diuresis.

In summary, our data suggest that the pressor response to systemic administration of U50,488H is mediated by two different mechanisms of action, first an activation of the sympathetic nervous system which is rapid but short-lived, followed by a more sustained release of vasopressin.

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