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Cardiovascular and respiratory actions of U50,488H in the unanaesthetized ovine foetus

Hazel H. Szeto *, Dunli Wu, Peter Y. Cheng, Yi Soong, Cindy C. Taylor, Jenny Yee

Department of Pharmacology, Cornell University Medical College, 1300 York Avenue, New York, NY 10021, USA

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Abstract

In an effort to evaluate the feasibility of κ-opioid receptor agonists for use in pregnancy, we have investigated the actions of U50,488H (*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide) on cardiovascular and respiratory control in the unanaesthetized ovine foetus. Intravenous administration of U50,488H (1.0 mg/kg) to the foetus resulted in an immediate increase in foetal blood pressure ($P < 0.0001$) and heart rate ($P < 0.0001$) which lasted 15 min, followed by a prolonged loss of heart rate variability for up to 3 h. There was also a significant suppression of foetal breathing movements for 2–3 h ($P < 0.008$). Pretreatment with naloxone (12 mg/h) completely blocked the hypertensive and tachycardiac response to U50,488H, but was unable to prevent the loss of variation in heart rate or respiratory depression. These data suggest that U50,488H can exert direct cardiovascular and respiratory actions in the ovine foetus via both opioid and non-opioid mechanisms. The naloxone-insensitive suppression of foetal breathing would severely limit the use of U50,488H as an obstetrical analgesic.

Keywords: Opioid; κ-Opioid receptor agonist; Pregnancy; Blood pressure; Heart rate

1. Introduction

Biochemical, pharmacological and molecular biological data all support the existence of multiple subclasses of opioid receptors with unique pharmacological profiles. This concept is of particular interest as it suggests the possibility of separating the desirable analgesic properties of opioid drugs from their unwanted side effects. There has been much interest in the development of κ-opioid receptor agonists as they appear to be free of many of the adverse effects of μ-opioid receptor agonists including constipation, respiratory depression and the development of tolerance and development (Freye et al., 1983; Young et al., 1984; Howell et al., 1990; Butelman et al., 1993).

κ-Opioid receptor agonists may also have particular advantage in the treatment of labour pain. The latter stages of pregnancy and parturition are associated with an increase in pain threshold that appears to be mediated by opioid receptors at the spinal cord level (Sander and Gintzler, 1987). This pregnancy-associated increase in pain threshold was effectively reduced by the κ-opioid receptor antagonist, nor-binaltorphimine, suggesting the involvement of spinal κ-opioid receptors in determining pain threshold during late pregnancy (Sander et al., 1988). These investigators further showed that the lumbar content of dynorphin A-(1–17) and dynorphin A-(1–8) is elevated during late pregnancy (Medina et al., 1993), and that intrathecal administration of dynorphin antibodies significantly reduced pain threshold in pregnant rats (Sander et al., 1989). These findings are all consistent with the proposal that a spinal cord dynorphin/κ-opioid receptor system is activated in late pregnancy.

Further subclassification of κ-opioid receptors has been proposed based on both ligand binding and functional data (Wollemann et al., 1993). The involvement of dynorphin in pregnancy-associated analgesia, its localization to the spinal cord, and its sensitivity to nor-binaltorphimine, suggest that κ_1-opioid receptor agonists, such as the benzacetamides, may be particularly effective as obstetrical analgesics. U50,488H (*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide) was the first benzacetamide

* Corresponding author. Tel.: 212-746-6232; fax: 212-746-8835; e-mail: hhszeto@mail.med.cornell.edu.

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to be introduced and was found to be equipotent to morphine in a variety of antinociceptive tests in mice and rats (Von Voigtlander et al., 1983). However, U50,488H has not been tested in pregnant animals. Other than its ability to inhibit progression of parturition in rats by reducing oxytocin secretion (Douglas et al., 1993), little is known about the effects of U50,488H on pregnancy outcome. U50,488H can be expected to cross the placenta with little difficulty and may result in pharmacological effects in the foetus. The present study investigated the direct actions of U50,488H on foetal cardiovascular and respiratory regulation in the late-term ovine foetus.

2. Materials and methods

2.1. Animal preparation

16 pregnant sheep with gestational ages ranging from 115 days to 143 days (term being approximately 145 days) were used in this study. 5 or more days prior to the study, chronic indwelling catheters and electrodes were implanted in foetal sheep in accordance with guidelines approved by the Institution for the Care and Use of Animals at Cornell University Medical College. Surgery was performed with the pregnant ewe under intrathecal lidocaine anaesthesia and supplemented with intravenous sodium pentobarbital. Details of the surgical procedure have been described previously (Szeto, 1983). Briefly, a polyvinyl catheter was placed in the foetal aorta for continuous recording of foetal blood pressure and blood sampling. Two polyvinyl catheters were placed in the foetal inferior vena cava, one for drug administration and the other for blood sampling. A pair of stainless steel electrodes was implanted subcutaneously in the foetus for recording of the electrocardiogram (EKG), and a pair of stainless steel electromyographic (EMG) electrodes was implanted in the foetal diaphragm for continuous recording of foetal breathing movements. All catheters and electrodes were tunneled subcutaneously to the maternal flank and stored in a pouch. Intraoperatively, 2 g of ampicillin was placed in the amniotic cavity and 1 g in the peritoneal cavity of the ewe. An estimate of foetal weight was obtained at the time of surgery.

2.2. Study design

To allow ample time for recovery from the surgical procedures, all drug experiments were performed at least 5 days after surgery. The studies were carried out with the ewe standing or lying quietly in a small experimental cart with free access to food and water. The ewe was allowed a period of 3 h to acclimate to the study conditions prior to drug administration. Continuous recording of foetal blood pressure, EKG and diaphragmatic EMG were obtained with a Gould 2800S analog recorder and appropriate amplifiers, and stored on FM tape (TEAC XR-310). Foetal heart rate was obtained either from the blood pressure or EKG recording by use of a Gould Biotach coupler. The incidence of foetal breathing was quantitated as the percent of time with breathing movements based on diaphragmatic EMG activity. Towards the end of the third hour, blood samples were obtained for determination of arterial blood gases and pH (Radiometer ABL30). Animals were included in the study only if foetal blood gases were within the normal range (PO_2 16–25 mm Hg, PCO_2 40–55 mm Hg, and pH 7.33–7.40).

U50,488H (RBI, Natick, MA; 0.5 or 1.0 mg/kg) or saline was administered to the foetus intravenously over 1.0 min. Foetal weight on the day of the experiment was calculated from the body weight estimated on the day of surgery based on a growth rate of 2% per day (Joubert, 1956). In five animals, naloxone (gift from Dupont-Merck Pharmaceutical Co., Wilmington, DE; 12 mg/h) was infused intravenously to the foetus starting 1 h prior to the administration of U50,488H, and maintained for a total of 4 h. Each foetus was allowed a minimum of 3 days between drug studies and only received each drug treatment once.

2.3. Data analysis

All data are presented as means ± 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, heart rate, and foetal breathing movements. 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 naloxone to modify the responses to U50,488H was evaluated using Friedman's two-way ANOVA with repeated measure (factors = treatment, time). Differences were considered significant when $P < 0.05$.

3. Results

3.1. Effects of U50,488H on foetal blood pressure and heart rate

Administration of saline ($n = 5$) or U50,488H (0.5 mg/kg; $n = 3$) had no effect on either foetal blood pressure or heart rate. A control polygraphic recording of foetal blood pressure and heart rate from one ovine foetus is illustrated in Fig. 1A. Intravenous administration of U50,488H (1.0 mg/kg) to the foetus resulted in an immediate increase in foetal blood pressure and heart rate which lasted approximately 15 min (Fig. 1B).

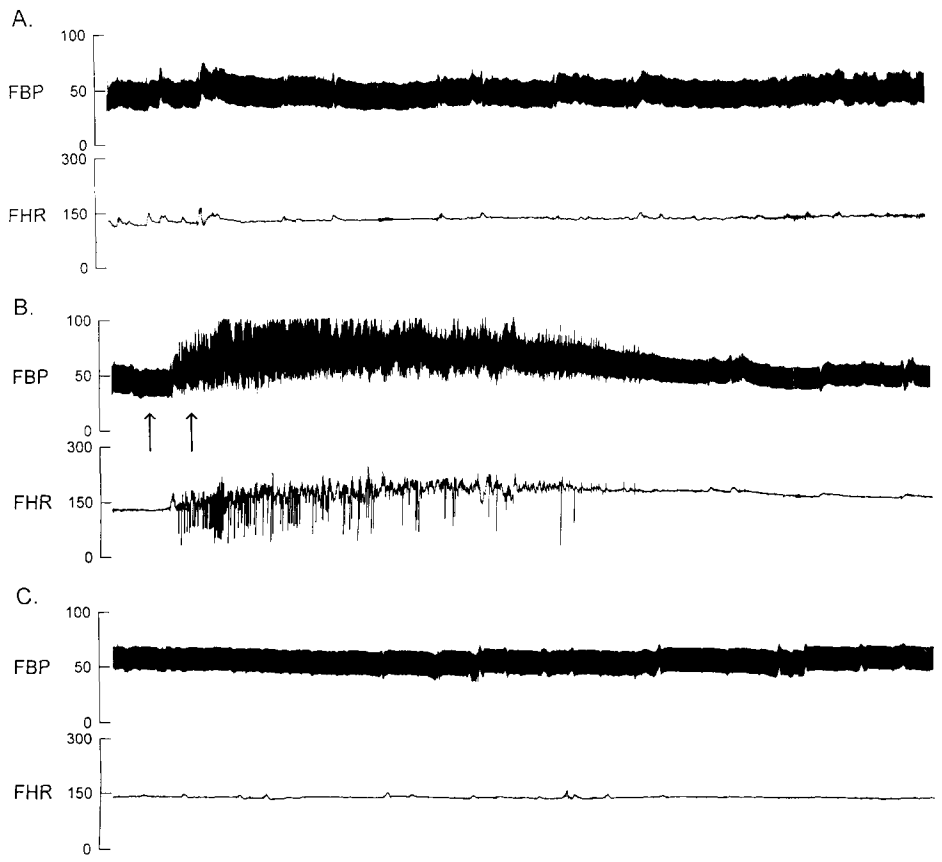

Fig. 1. Polygraphic recording from a representative foetus showing foetal blood pressure (FBP) and foetal heart rate (FHR) before (A), immediately after (B) and 2 h after (C) the i.v. administration of U50,488H (1.0 mg/kg) to the foetus. The administration of U50,488H is illustrated by the ↑ ↑. Each panel represents 20 min.

This effect of U50,488H on foetal cardiovascular function was observed in all animals studied. Fig. 2 summarizes this immediate effect of U50,488H on mean blood pressure and heart rate in 10 animals. The effect of U50,488H on mean foetal blood pressure was highly significant ($\chi^2 = 71.9$; $P < 0.0001$), and post-hoc comparison revealed that mean blood pressure was significantly elevated compared to pre-drug values from 1 to 15 min ($P < 0.05$). Similarly, the effect of U50,488H on foetal heart rate was also highly significant ($\chi^2 = 60.5$; $P < 0.0001$), and heart rate remained significantly elevated from control for up to 15 min. This immediate response was then followed by a prolonged decrease in foetal heart rate variability which lasted 2–3 h (Fig. 1C). Pretreatment with naloxone completely blocked the immediate effects of U50,488H on foetal blood pressure and heart rate (Fig. 2). Two-way analysis of variance confirmed that naloxone pretreatment significantly altered the blood pressure ($F = 20.9$; $P = 0.001$) and heart rate ($F = 13.8$; $P = 0.005$) response to U50,488H. Naloxone, however, was unable to block the later reduction in foetal heart rate variability.

3.2. Effect of U50,488H on foetal breathing movements

Under control conditions, foetal breathing movements occurred intermittently in all animals and were present 48.2 ± 9.1% of total recording time. Administration of U50,488H resulted in a significant reduction in foetal breathing movements which lasted for almost 3 h ($\chi^2 = 13.5$; $P = 0.009$) (Fig. 3). There was a complete suppression of foetal breathing movements in

Table 1
Effects of U50,488H on foetal arterial blood gases

Drug	pH		PCO_2		PO_2	
	Control	60 min	Control	60 min	Control	60 min
U50,488H	7.32 ± 0.01	7.33 ± 0.01	56.7 ± 1.3	55.6 ± 1.4	31.3 ± 2.5	29.7 ± 2.5
Naloxone + U50,488H	7.3 ± 0.01	7.34 ± 0.01	56.2 ± 1.2	55.3 ± 1.6	27.0 ± 2.6	27.4 ± 1.9

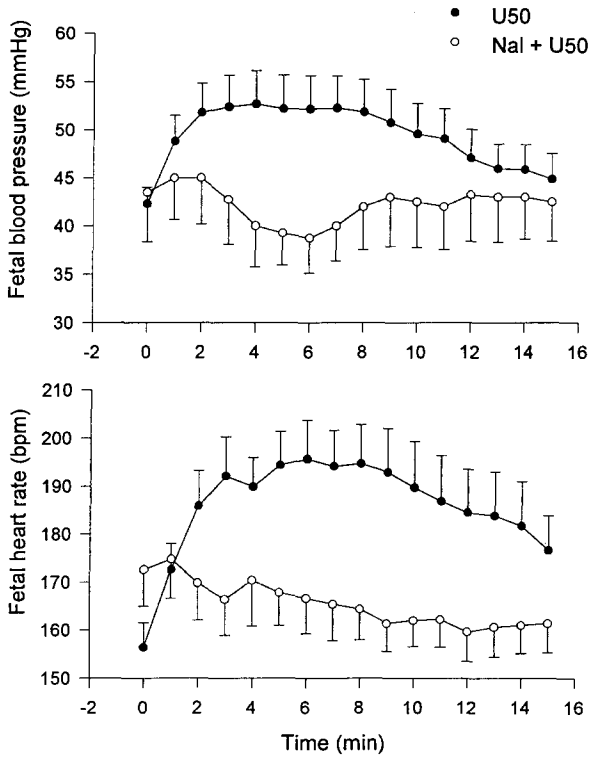

Fig. 2. Effects of U50,488H (1.0 mg/kg, i.v.) on foetal mean blood pressure and foetal heart rate in the absence (●) and presence (○) of naloxone (12 mg/h). Data are presented as means ± S.E. ($n = 10$).

some animals. Saline had no effect on the incidence of foetal breathing movements. Naloxone pretreatment had no effect on the suppression of foetal breathing movements by U50,488H, and foetal breathing remained significantly reduced compared to control ($\chi^2 = 7.6$; $P = 0.008$) (Fig. 3).

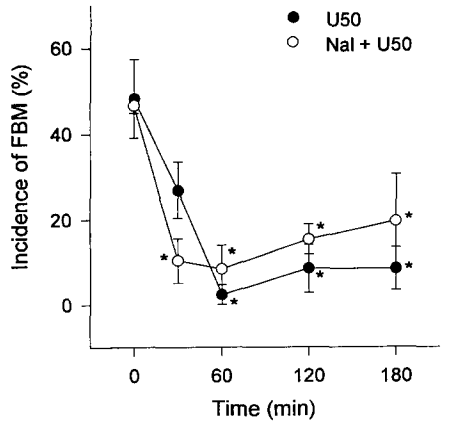

Fig. 3. Effects of U50,488H (1.0 mg/kg, i.v.) on the incidence of foetal breathing movements in the absence (●) and presence (○) of naloxone (12 mg/h).

3.3. Effect of U50,488H on foetal arterial blood gases

The effects of U50,488H on foetal PO_2, PCO_2 and pH are summarized in Table 1. There was no significant change in any of the parameters.

4. Discussion

U50,488H is lipid soluble and can be expected to distribute across the placenta readily and reach significant levels in the foetus. The effects of κ-opioid receptor agonists on the foetus have not been investigated. κ Binding sites have been demonstrated both in the brain as well as the spinal cord in neonatal animals, and κ sites were the first of the three major subtypes of opioid receptors to reach adult levels at 7–14 days after birth in the rat (Petrillo et al., 1987). Binding studies with the κ_1 ligand U69,593 actually revealed higher levels of binding in the spinal cord of 9–16 day old rats when compared to the adult, and this presence of binding sites was correlated with functional response in both in vivo and in vitro tests (Allerton et al., 1989). Furthermore, the actions of κ-opioid receptor agonists on antinociception and prolactin release are apparent by 3–10 days in rat pups, and mature earlier than the response to μ-opioid receptor agonists (Bero et al., 1987; Barr et al., 1992). Although binding data are not available in the human or sheep, the development of κ-opioid receptors can be expected to be well on the way in the late-term human or ovine foetus.

The results of the present study show that U50,488H can have direct actions on both cardiovascular and respiratory function in the ovine foetus. Intravenous administration of U50,488H to the foetus resulted in a rapid and highly significant increase in foetal blood pressure and heart rate. This is in contrast to most findings in the adult, where intravenous U50,488H has been reported to reduce arterial blood pressure and heart rate (Hall et al., 1988; Pugsley et al., 1992). This discrepancy may be due to the presence of anesthetic agents in those adult studies. U69,593 apparently had no effect on blood pressure or heart rate when administered i.c.v. to conscious rabbits (May et al., 1989), and i.v. administration of U62,066E also had no effect on arterial pressure or heart rate in the human (Rimoy et al., 1994).

The effects of U50,488H on foetal blood pressure and heart rate appear to be mediated by specific opioid receptors as they were completely blocked by naloxone pretreatment. The more selective κ-opioid receptor antagonist, nor-binaltorphimine, was not used in the present study because its extremely long duration of action makes it quite impractical in our animal model

(Horan et al., 1992) and recent studies suggest that it can act as an antagonist at multiple opioid receptor subtypes (Spanagel et al., 1994). We are, however, quite confident that this dose of U50,488H was acting selectively at κ-opioid receptors since i.v. administration of μ-opioid ([D-Ala^2,MePhe^4,Gly^5-ol]enkephalin) or δ-opioid ([D-Pen^2,D-Pen^5]enkephalin) receptor agonists to the ovine foetus did not result in significant changes in arterial pressure or heart rate (unpublished data). A significant pressor effect to U50,488H has been reported in conscious adult rats after direct injection into the nucleus tractus solitarii, suggesting a central mechanism of action (Carter and Lightman, 1985). This pressor response was not modified by α-adrenoceptor blockade, but was associated with an increase in circulating vasopressin levels and was blocked by a vasopressin antagonist. The role of vasopressin was further confirmed by the lack of a pressor response to U50,488H in vasopressin-deficient Brattleboro rats (Carter and Lightman, 1985). The mechanism for the pressor response observed in the ovine foetus has not yet been determined, but our laboratory has recently found that this dose of U50,488H produced a significant release of adrenocorticotropin which could be attenuated by a vasopressin antagonist (unpublished data). It is therefore quite likely that vasopressin may play a role in the vasoconstrictive action of U50,488H in the ovine foetus.

In the anaesthetized adult rat, the pressor effect of U50,488H was accompanied by a decrease in heart rate, consistent with a baroreflex response (Carter and Lightman, 1985). In the ovine foetus, however, we observed a concomitant increase in foetal heart rate, suggesting either a reduction in baroreceptor gain, exaggerated sympathetic activity, or a direct cardiostimulatory action of U50,488H. A direct action of U50,488H on baroreceptor gain is supported by the prolonged loss of foetal heart rate variability after the initial hypertensive and tachycardiac response.

The loss of foetal heart rate variability coincides with the suppression of foetal breathing movements. Foetal breathing movements normally occur intermittently and in clusters ranging from 10 to 30 min in duration. During these intervals of breathing movements, there is an increase in heart rate variability which is similar to respiratory sinus arrhythmia in postnatal life (Dawes et al., 1972). The suppression of foetal breathing movements by U50,488H was unexpected as most studies in adult animals have found little or no effect of U50,488H on respiration (Castillo et al., 1986; May et al., 1989; Howell et al., 1990; France et al., 1994). U50,488H has even been reported to antagonize the respiratory depression caused by μ-opioid receptor agonists (Dosaka-Akita et al., 1993). The mechanism behind this significant decrease in foetal breathing movements is not known, and it was not blocked by naloxone. It is unlikely that the dose of naloxone was too low to block κ-opioid receptors as it was certainly high enough to completely block the vasoconstrictive actions of U50,488H. Although we have previously shown that the μ-opioid receptor agonist DAMGO ([D-Ala^2,MePhe^4,Gly^5-ol]enkephalin) also suppresses foetal breathing movements, it is unlikely that this dose of U50,488H was acting at μ-opioid receptors since this dose of naloxone is more than adequate in blocking the actions of DAMGO on foetal breathing (Szeto et al., 1995). Foetal breathing movements are known to be modulated by arterial blood gases, with hypoxia producing a decrease in foetal breathing movements and hypercapnia stimulating foetal breathing movements (Boddy et al., 1974). U50,488H may cause foetal hypoxia by decreasing placental blood flow or oxygen delivery to the foetus, but our data show that the respiratory depression is not secondary to foetal hypoxia. Finally, U50,488H has been reported to have non-opioid action (Hayes et al., 1988). Regardless of the mechanisms involved, the depression of foetal breathing movements severely limits the potential use of U50,488H as an obstetric analgesic.

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