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β-Adrenoceptive responses in the unanaesthetized ovine foetus

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Summary

1. Isoprenaline injection into either the unanaesthetized ovine foetus or the pregnant ewe produced a transient tachycardia and hypotension in either the ewe or the foetus. No evidence was obtained for placental transfer, in either direction, of pharmacologically active isoprenaline.

2. Propranolol, when given to the ewe intravenously, produced bradycardia and increased pulse pressure and inhibited the response of both the ewe and her foetus to isoprenaline. Propranolol, when given to the foetus intravenously, produced bradycardia and increased pulse pressure in both the foetus and the ewe, but only the foetal response to isoprenaline was inhibited. These data demonstrated that propranolol crossed the ovine placenta in both directions in a pharmacologically active form.

3. Dose-heart rate curves of the foetus and pregnant ewe to isoprenaline and the shift to the right of the isoprenaline dose-response curves by propranolol were similar in both the ewe and the foetus.

4. Notwithstanding the similarities between the ewe and foetus in their responses to isoprenaline or propranolol and in the antagonism of isoprenaline by propranolol, the duration of blockade following propranolol administration to the ewe was 2 to 3 times longer in the foetus compared with the ewe.

5. Measurement of blood levels of propranolol showed that the maximum concentration of propranolol in foetal plasma was only 5% of that in the pregnant ewe when propranolol was infused into the ewe ; the rate of clearance of propranolol was similar from the foetal and maternal plasma.

6. From these data the long duration of β-adrenoceptor blockade in the ovine foetus by propranolol cannot be fully explained. However, these data serve as examples of the dangers involved when extrapolating pharmacological actions of drugs on the foetus purely from data on foetal plasma levels of the drug.

7. The data suggest that multiple doses of propranolol, given to maintain a β-adrenoceptor blockade in the mother, could result in serious cumulative effects in the foetus.

Introduction

Blockade of the β-adrenoceptor system with drugs has been proposed for the treatment of a variety of conditions such as angina pectoris, cardiac arrhythmias,

endotoxic shock, and hypertension (Epstein & Braunwald, 1966a, b ; Berk, Hagen, Beyer, Gerber & Dochat, 1969 ; Fitzgerald, 1969). In addition to the above uses, the β-adrenoceptor blocking agent, propranolol, has been utilized, together with catecholamines, to control uterine contractions during pregnancy (Eskes, Stolte, Seelen, Moed & Vogelsang, 1965 ; Stolte, Eskes, Seelen, Moed & Vogelsang, 1965 ; Barden & Stander, 1968). Preliminary observations from this laboratory (Van Petten & Willes, 1968) and others (Joelsson & Barton, 1969) indicated that propranolol crossed the ovine placenta in a pharmacologically active form and produced a β-adrenoceptor blockade in the foetus. The present experiments were conducted to determine more precisely the type of blockade and duration of action of propranolol in the foetus and the relationship between these actions and those in the ewe.

Methods

Horn Dorset and Western Whiteface sheep, 1 to 4 years of age, with known breeding dates, were used throughout the study. Arterial and venous cannulas and electrocardiograph (e.c.g.) electrodes were chronically implanted into twenty ovine foetuses between 100 and 120 days of gestation as previously described (Willes, Manns & Boda, 1969 ; Willes, Van Petten & Truelove, 1970). The carotid artery and jugular vein of the pregnant ewes were chronically cannulated. In experiments with pregnant ewes or newborn lambs, needle-type e.c.g. electrodes were inserted under the skin at both ends of the sternum. A multi-channel physiological recorder (Hewlett Packard 7718A) was used to record arterial blood pressure in mm Hg (1 mm Hg$\equiv$1·333 mbar), by means of pressure transducers, and e.c.g. simultaneously in the non-anaesthetized ewe and foetus ; cardiotachometers were used to compute the heart rates from the e.c.g. In the lamb only e.c.g. and heart rate were monitored.

Isoprenaline dose-response curves and effect of propranolol

Dose-response curves were constructed from the peak positive chronotropic responses to varying doses of isoprenaline injected intravenously into the pregnant ewe and into the foetus. At least five replicate experiments were conducted at each isoprenaline dose. Foetal and maternal heart rates and blood pressures were monitored at 0 min (pre-injection) and 0·5, 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 min following each isoprenaline dose. At least 30 min elapsed between doses of isoprenaline. In several experiments isoprenaline was injected alternately into the pregnant ewe or her foetus to determine whether isoprenaline crossed the ovine placenta in either direction in a pharmacologically active form. All foetal doses of drugs were based on estimated foetal body weights (Stephenson, 1959).

To compare the degree of the β-adrenoceptor blockade produced by propranolol in the ovine foetus with that in the ewe, propranolol (1·0 mg/kg body weight) was infused over a 10 min period into the pregnant ewe. The peak chronotropic response produced by isoprenaline (5·0, 10 and 25 μg/kg body weight) in the ewe and foetus was measured 0·5 h after the propranolol was given. Only one dose of isoprenaline was given after propranolol in each experiment of this type and six experiments were conducted at each dose of isoprenaline.

Duration of blockade by propranolol

Propranolol was administered intravenously to (1) the pregnant ewe at doses of 1·0, 0·5 and 0·1 mg/kg maternal body weight, (2) the foetus at a dose of 1·0 mg/kg foetal body weight, or (3) the newborn lamb (1 to 48 h post-partum) at a dose of 1·0 mg/kg body weight. The effect of propranolol itself on heart rate and blood pressure was monitored for 30 min before testing the β-adrenoceptor blockade.

The duration of the β-adrenoceptor blockade produced by the administration of propranolol to the pregnant ewe was measured by determining the tachycardia produced by the intravenous administration of isoprenaline 1 μg/kg simultaneously to the ewe and to the foetus at various times after injection of propranolol into the ewe ; these responses were compared with the isoprenaline responses before administration of propranolol. The duration of β-adrenoceptor blockade produced after propranolol injection into the newborn lamb or into the foetus was assessed in a similar manner.

Plasma propranolol concentrations

Propranolol (1 mg/kg) was infused intravenously into the pregnant ewe over a 10 min period in ten experiments. Blood samples (4 ml) were withdrawn from (*a*) the carotid artery of the ewe at 1 min intervals until 10 min, then at 2 min intervals until 30 min and (*b*) from the abdominal aorta of the foetus at 2 min intervals until 30 min after the start of propranolol infusion. The propranolol concentration in the plasma was measured spectrophotofluorometrically using the method of Stock & Westermann (1965).

Results

Effects of isoprenaline

Isoprenaline injected into either the ewe or the foetus resulted in an increase in heart rate and decrease in blood pressure but little change in pulse pressure (Fig. 1). The maximum heart rate obtained following 1 μg/kg isoprenaline was similar in the ewe and the foetus, although the maximum response was delayed in the foetus until about 2 min after injection of isoprenaline (Fig. 1). Isoprenaline (1 μg/kg) produced a tachycardia in the newborn lamb which was similar to that observed in the ewe. Isoprenaline injection into the ewe, at doses up to 5 μg/kg, did not produce a measurable response in the foetus ; similarly, isoprenaline injection into the foetus produced no detectable response in the ewe.

The dose-response curves of the ewe and the foetus to isoprenaline were similar when the peak heart rate was used as a measure of the response (Fig. 2). However, the percentage increase in heart rate following a given dose of isoprenaline was greater in the ewe than in the foetus (Fig. 2), since the control heart rates (time 0) were much higher in the foetus (compare in Tables 1 and 2). No obvious differences were observed between the responses of foetuses of different gestational ages to isoprenaline, indicating that the β-adrenoceptor system of the foetal heart was fully developed as early as 100 days of gestation (the earliest time tested).

Effects of propranolol

Intravenous infusion of propranolol into the pregnant ewe resulted in a decrease in heart rate and increase in pulse pressure in both the ewe and the foetus (Table 1).

When propranolol was infused directly into the foetus, similar but smaller changes in the heart rate and pulse pressure were observed in both the ewe and the foetus (Table 2). Bradycardia was also observed following propranolol administration to newborn lambs (Table 2).

Antagonism of isoprenaline by propranolol

Propranolol, when administered to the pregnant ewe, effectively inhibited the tachycardia and hypotension typically produced by isoprenaline in both the ewe and the foetus. The administration of 5, 10 and 25 μg/kg of isoprenaline to the ewe and to the foetus following infusion of 1 mg/kg body weight propranolol into the ewe resulted in a parallel shift to the right of the isoprenaline dose-response curves (Fig. 3). Larger doses of isoprenaline (>25 μg/kg) frequently produced cardiac arrhythmias and even cardiac arrest, especially in the foetus. Because of this problem, data for the upper portion of the shifted dose-response curves could not be obtained. No apparent differences were observed between the ewe and foetus with respect to the shift in isoprenaline dose-response curve (Fig. 3).

Propranolol infusion into the ewe at doses of 0·1, 0·5 and 1·0 mg/kg maternal body weight produced a β-adrenoceptor blockade which lasted 1, 2 and 4 h in the

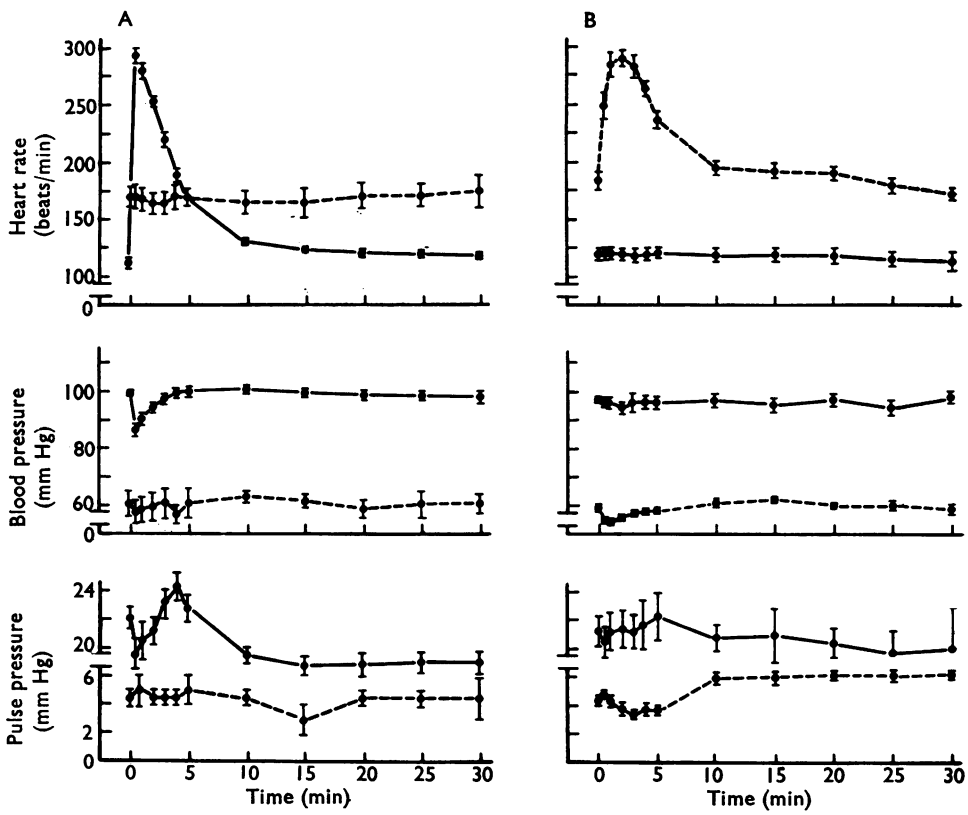

FIG. 1. Heart rate (beats/min), blood pressure (mm Hg) and pulse pressure (mm Hg)± standard errors for ewe (●——●) and foetus (●- - -●) following intravenous injection of isoprenaline (1 μg/kg) into the ewe (A) and the foetus (B).

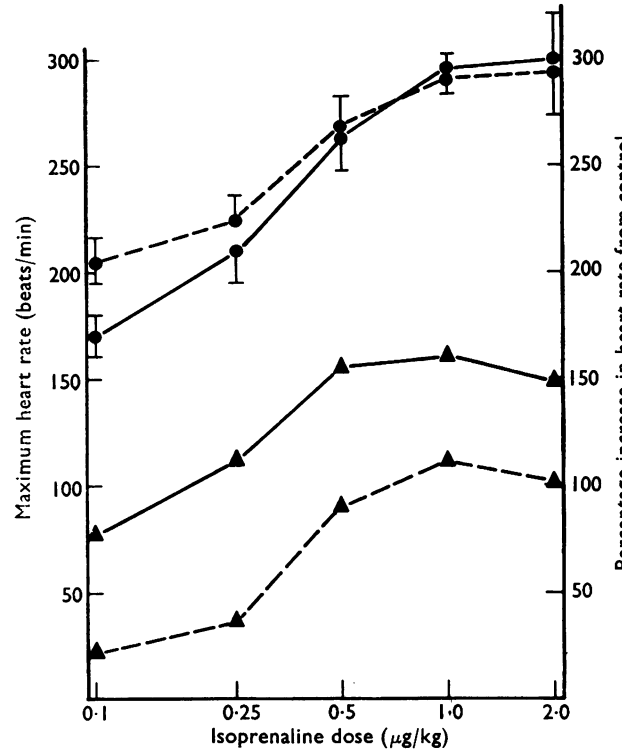

FIG. 2. Maximum heart rate (beats/min ± standard error) of the ewe (●——●) and foetus (●- - - -●) after intravenous injection of isoprenaline. Percentage increase in heart rate from control for the ewe (▲——▲) and foetus (▲- - - -▲) after intravenous injection of isoprenaline calculated from maximum heart rate data.

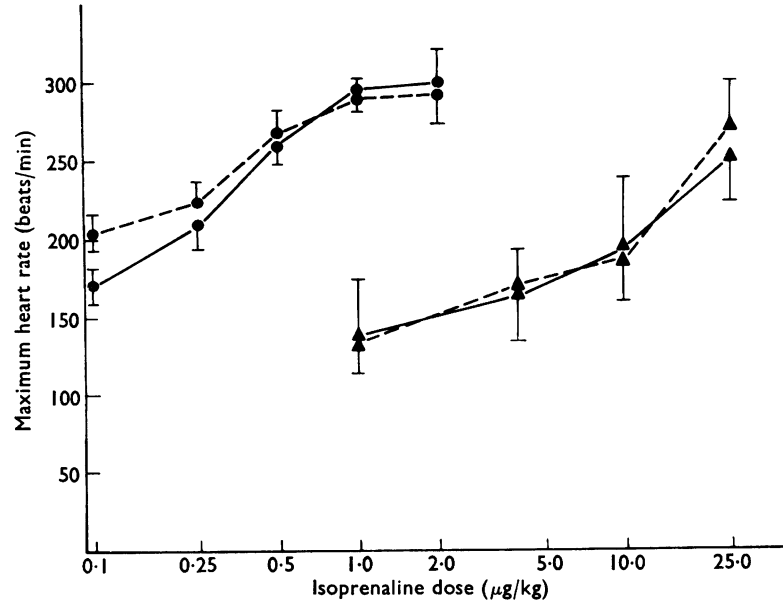

FIG. 3. Maximum heart rate (beats/min ± standard error) produced by intravenous injection of isoprenaline into the ewe (●——●) and foetus (●- - - -●) in absence of propranolol and into the ewe (▲——▲) and foetus (▲- - - -▲) after intravenous infusion of propranolol 1 mg/kg body weight into the ewe. Only one injection of isoprenaline was given with any one dose of propranolol to avoid tachyphylaxis and toxic effects of repeated high doses of isoprenaline.

TABLE 1. *Heart rate (HR), blood pressure (BP) and pulse pressure (PP)±standard errors of the dam and foetus at various time intervals following intra-jugular infusion of propranolol (1 mg/kg) into the pregnant ewe.*

		Time after propranolol injected (min)										
Subject	Variable	0	2	5	10	20	30	60	120	240	480	600
Dam (31)	HR	102±4	86±3	81±2	79±2	79±2	85±3	85±3	95±4	100±5	116±4	118±5
	BP	97·7±1·5	97·0±1·4	97·7±1·5	96·5±1·6	95·5±1·2	93·5±3·0	98·3±2·4	95·0±2·3	99·4±3·2	103±1·8	104±2·5
	PP	20·0±0·9	22·3±0·9	23·2±0·9	24·6±0·3	25·2±0·8	25·0±1·8	23·7±1·3	23·6±1·6	25·7±1·5	21·3±1·6	18·2±3·7
Foetus (18)	HR	182±6	180±8	164±8	154±5	154±4	154±7	155±7	161±6	177±5	180±6	183±9
	BP	59·4±1·5	59·5±2·1	60·9±1·6	63·3±2·1	58·7±1·4	63·5±4·9	60·4±1·4	60·3±2·1	61·0±1·7	60·8±1·2	60·0±3·6
	PP	4·3±0·5	5·2±0·7	6·2±0·8	5·6±0·9	4·7±0·6	4·8±0·5	5·3±0·5	5·3±0·7	5·5±0·3	5·8±0·6	6·3±1·5

TABLE 2. *A: Heart rate (HR), blood pressure (BP) and pulse pressure (PP)±standard errors of the dam and foetus at various time intervals following intravenous infusion of propranolol (1 mg/kg) into the foetus. B: Heart rate (HR)±standard error of lambs following intra-jugular injection of propranolol (1 mg/kg) into the lamb*

		Time after propranolol injected (min)								
Subject	Variable	0	2	5	10	20	30	60	120	240
(A) Dam (8)	HR	123±7	120±6	121±8	115±8	110±6	116±3	111±10	116±10	125±6
	BP	103±3·1	103±3·2	98·5±4·7	103±3·2	106±8·4	100±5·8	107±1·7	97·0±2·0	107±3·5
	PP	22·5±2·5	20·0±1·3	22·3±2·3	21·3±1·3	24·0±1·5	22·7±1·5	23·7±1·3	23·5±3·5	25·0±5·0
Foetus (8)	HR	162±8	150±5	151±5	152±5	151±7	149±9	151±9	163±9	178±12
	BP	58·3±5·2	60·0±2·3	61·3±2·6	65·7±3·3	60·3±2·4	63·0±2·1	56·0±1·0	58·0±3·5	57·0±6·0
	PP	4·7±1·8	6·3±1·5	6·0±1·5	6·3±1·5	5·3±0·9	5·3±1·5	5·5±0·5	5·0±1·2	6·0±1·0
(B) Lamb (10)	HR	219±14	189±10	185±12	184±12	184±13	181±14	191±9	205±15	196±11

ewe respectively and 2, 4 and 10 h in the foetus respectively (Fig. 4). The administration of propranolol (1 mg/kg foetal body weight) directly to the foetus effectively blocked the response of the foetus to isoprenaline for 3–4 h but did not inhibit the response of the pregnant ewe to isoprenaline (Fig. 5A). The response of the newborn lamb to isoprenaline was effectively inhibited by propranolol (1 mg/kg); the duration of the β-adrenoceptor blockade was 3 to 4 h (Fig. 5B).

Plasma levels of propranolol

The measurement of propranolol concentrations in foetal and maternal plasma following propranolol infusion into the ewe demonstrated that propranolol crossed the ovine placenta; the maximum concentration achieved in the foetal plasma (at 14 min) was about 5% of the maximum achieved in the ewe (at 8 min) (Fig. 6). At

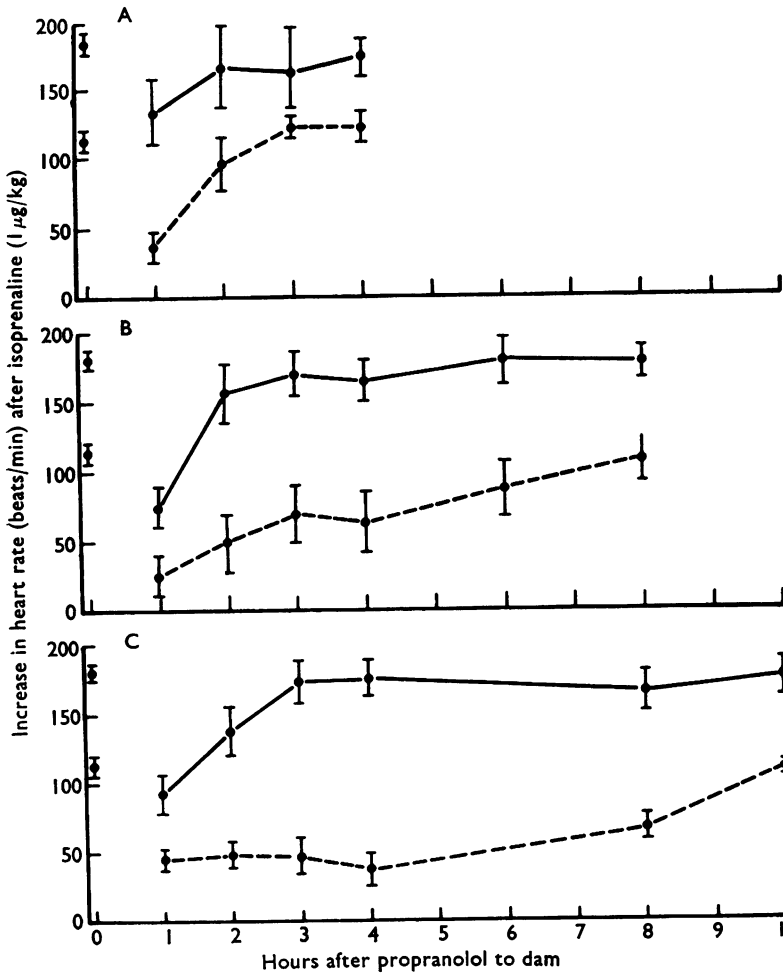

FIG. 4. Increase in heart rate (beats/min ± standard error) of the ewe (●——●) and foetus (○- - -○) produced by intravenous injections of isoprenaline (1 μg/kg) at various time intervals after intravenous infusion of propranolol into the ewe at 0·1 mg/kg (A), 0·5 mg/kg (B) and 1·0 mg/kg (C). The control responses to isoprenaline before propranolol infusion are shown at time 0 as 181 ± 5 and 110 ± 8 beats/min for ewe and foetus respectively.

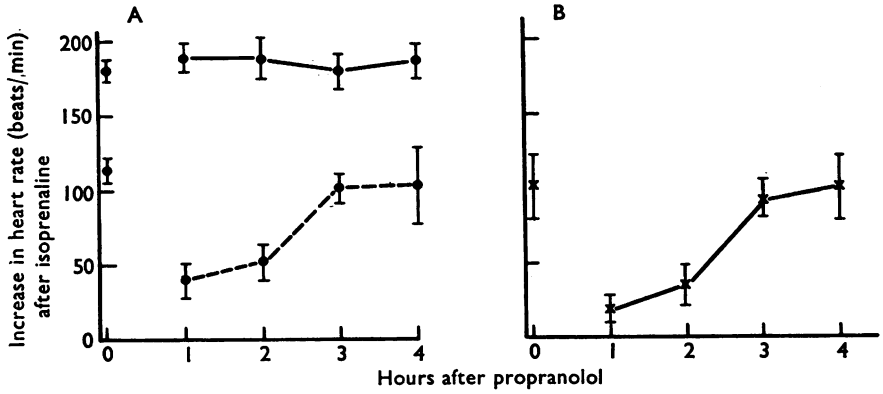

FIG. 5. A: Increase in heart rate (beats/min ± standard error) of the ewe (●——●) and foetus (●- - - -●) produced by intravenous injections of isoprenaline (1 μg/kg) at various times after intravenous infusion of propranolol (10 min) into the foetus, at 1 mg/kg foetal body weight. The control responses to isoprenaline before propranolol infusion are shown at time 0 as 181 ± 5 and 110 ± 8 beats/min for ewes and foetuses respectively. B: Increase in heart rate (beats/min ± standard error) of newborn lambs produced by intravenous injections of isoprenaline (1 μg/kg) at various times after intravenous injection of propranolol into lambs at 1·0 mg/kg body weight. The increase in heart rate of lambs to isoprenaline before propranolol injection is shown at time 0 as 101 ± 15 beats/min.

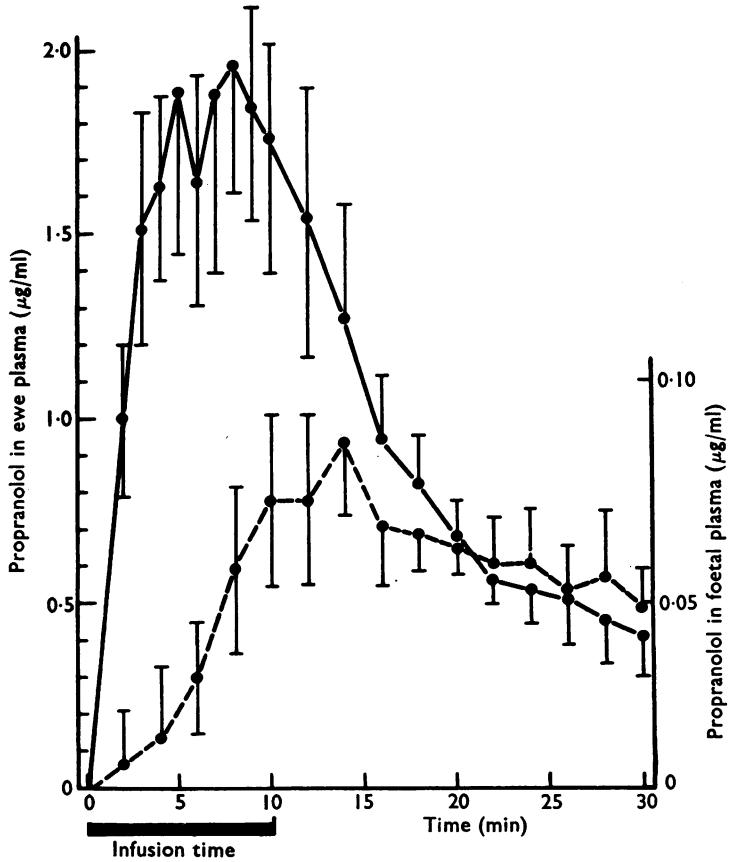

FIG. 6. Propranolol concentration (μg/ml ± standard error) in ewe (●——●) and foetal (●- - - -●) plasma during and after propranolol infusion into pregnant ewes.

equilibrium (20 min) the foetal concentration was still only 10% of that in the ewe and thereafter the rate of disappearance from both circulations was similar (Fig. 6). Propranolol concentrations in the ewe and foetal plasma following propranolol infusion into the foetus (1 mg/kg foetal body weight) were below the level of sensitivity of the propranolol assay.

Discussion

Tachycardia and hypotension were observed following isoprenaline administration to the unanaesthetized ovine foetus. This response was similar to that following isoprenaline administration to the ewe and to the response observed in the anaesthetized foetus (Campbell, Dawes, Fishman & Hyman, 1967; Assali, Bekey & Morrison, 1968; Dawes, 1968). When isoprenaline was administered to the ewe no response was observed in the foetus; similarly, isoprenaline injection into the foetus did not elicit a response in the ewe. Thus, at the doses used, no evidence was obtained for placental transfer, in either direction, of a pharmacologically active form of this drug.

In either the pregnant ewe or foetus propranolol produced bradycardia and an increased pulse pressure, but no significant hypotensive effect was observed confirming reports on other unanaesthetized animals (Kontos & Lower, 1969). Propranolol crossed the ovine placenta and, when given to the ewe, effectively inhibited the response of both the ewe and foetus to isoprenaline. When propranolol was administered to the foetus, a bradycardia resulted in both the foetus and ewe but only the foetal response to isoprenaline was inhibited; this failure to detect β-adrenoceptor blockade in the ewe was probably due to the large dilution of the foetal dose (based on foetal body weight) of propranolol in the maternal system.

The dose-response curves of the ewe and foetus to isoprenaline were very similar, indicating that the reactions between isoprenaline and the β-adrenoceptor system were quantitatively similar in terms of the dose required to produce a peak increase in heart rate in the ewe and foetus. The apparently parallel shift of the isoprenaline dose-response curves to the right after propranolol was of the same magnitude in both the ewe and foetus, indicating a competitive type blockade in both cases. Thus, these experiments demonstrated that the affinity of the β-adrenoceptor for isoprenaline and propranolol was very similar in the ewe and foetus.

Notwithstanding the similarities between the ewe and foetus in the responses to isoprenaline or propranolol and in the antagonism of isoprenaline by propranolol, the duration of the β-adrenoceptor blockade following administration of propranolol to the ewe was 2 to 3 times longer in the foetus compared with the ewe. This suggested that the longer duration of action of propranolol in the foetus might be due to sustained high levels of propranolol in the foetus. However, measurement of propranolol concentrations in foetal and maternal plasma following propranolol administration to the ewe demonstrated that the maximum propranolol concentration in foetal plasma was only 5% of that observed in the ewe; the clearance rate for propranolol was similar in both systems. Thus the difference in plasma levels of the drug would have suggested that the duration of blockade by propranolol should have been shorter in the foetus rather than longer, and that the degree of β-adrenoceptor blockade should have been less in the foetus rather than the same as in the ewe. The short duration of β-adrenoceptor blockade in the foetus found following

the administration of propranolol to the foetus may have been due to rapid placental transfer and dilution of this small mass of drug in the maternal circulation. In these latter experiments the levels of propranolol in the foetal and maternal circulation were undetectable with the method used.

It is known that propranolol is rapidly taken up by tissues, so that the plasma level of propranolol may not correlate with the magnitude or duration of β-adrenoceptor blockade (Stock & Westermann, 1965). Since tissue levels of propranolol were not measured in these experiments, the possibility cannot be excluded that greater non-specific binding of propranolol by foetal tissues or by the placenta was responsible for the longer duration of action of propranolol in the foetus following administration of the drug to the ewe. In addition, the hypothesis that propranolol may undergo bio-degradation in the foetal-placental unit to a metabolite having different properties, although unlikely because of the similar type of blockade in the ewe and foetus, cannot be completely discounted. Nevertheless, these data exemplify the danger of extrapolating pharmacological actions of drugs on the foetus purely from data on foetal plasma levels of the drug.

Despite the uncertainty as to the precise mechanism whereby propranolol given to the ewe produced a three-fold longer duration of action in the foetus, it seems apparent that multiple doses given at suitable intervals to maintain a β-adrenoceptor blockade in the mother could result in serious cumulative effects on the foetus.

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