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(19)

(54) DRUGS FOR INDUCTION OF LABOUR, FACILITATION OF FERTILISATION AND INCREASING MILK DELIVERY IN NON-HUMAN MAMMALS, PARTICULARLY FARM ANIMALS

(71) We, Československá akademie věd, a Czechoslovak Corporation, of No. 3 Národní, Praha 1, Czechoslovakia, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The subject of this invention is a pharmacological means of inducing labour, facilitating conception and increasing milk delivery in non-human mammals, particularly farm animals.

It is known from the literature that the natural hormone oxytocin produces contractions of the uterus in the last third of pregnancy, as well as contractions of the oviducts and the collecting ducts of the mammary gland with a resulting ejection of milk. The natural hormone has, however, two disadvantages: a) the actions following separate injections is very short-lived, so that to induce labour in a cow it must be injected at 30-minute intervals, which is difficult and sometimes impossible for the veterinary physician on practical grounds. In addition, natural oxytocin also produces vaso-constriction in the uterus, which is of disadvantage to the foetus as well as to nidation of a fertilised egg. It would be of advantage to have an oxytocic drug which would, on a single injection, result in an oxytocic response lasting for several hours. Analogues of oxytocin have been described with a prolonged oxytocic activity, particularly 1-deamino-1,6-dicarba-oxytocin (Kobayashi A., Hase S., Kiyoi R., Sakakibara S.: Bull. Chem. Soc. Japan *42*, 3491 (1969); Walter R., Yamanaka Y., Sakakibara S.: Proc. Nat. Acad. Sci. US *71*, 1901 (1974)), however dicarba-analogues, in which both sulphur atoms have been supplanted by methylene groups, although they have prolonged activity, show such low potencies that very large doses would be required (Barth T., Krejčí I., Kupková B., Jošt K.: Europ. J. Pharmacol. *24*, 183 (1973); Barth T., Krejčí I., Vaněčková J., Jošt K.: Europ. J. Pharmacol. *25*, 67 (1974)).

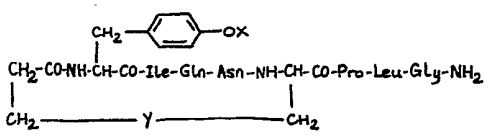
There is also a short-acting oxytocin analogue, [2-Tyr(OMe)] — oxytocin, which in comparison with the natural hormone has one order of ten lower contractile activity on the myometrium, but its vasoconstrictor activity in the same organ is decreased by two orders of ten (Hodr J., Štembera Z.K., Brotánek V., Rudinger J., Vondráček J.: The influence of Methyloxytocin on glycide metabolism of mother and foetus. Intrauterine Dangers to the Foetus. Excerpta Medica, Amsterdam 1966, p. 445) so that if 10 times as much analogue as parent hormone is administered the same expulsive force from the uterus can be attained without a toxic, hypoxic effect on the foetus. This short-acting oxytocin analogue is commercially produced and in clinical use (Methyloxytocin SPOFA) and its effectiveness has been demonstrated (Bärtschi R., Hüter J., Römer V.M.: Geburtshilfe und Frauenheilkunde *32*, 826 (1972)). Just as with oxytocin, induction of labour with this analogue requires a continuous infusion and careful following of uterine contractions and cardiac action of the foetus in order to prevent damage to the latter.

It is an object of this invention to provide a drug which after application would maintain its activity for a long period without need to repeat the application and would enable to control the delivery so that it would be accomplished at a required time.

It is another object of this invention to facilitate conception, particularly in case of artificial insemination.

A still further object is to increase milk delivery.

The subject matter of this invention is concerned with the use of a long-acting structural analogue of oxytocin, in which the disulphide bridge is replaced by a thioether one, as shown in the general formula I:



where X represents H or CH_3 and Y represents $—CH_2—S—$ or $—S—CH_2—$. The dosage varies from 10 ng to 100 μg/kg body wt. It has now been determined that the above substances can be used in particular for:

1) induction of labour in pregnant non-human animals;

2) facilitation of conception in non-human animals after artificial insemination; and

3) milk ejection in dairy animals.

These analogues of 1-deamino-oxytocin were prepared by classical synthetic methods in solution (Jošt K.: Collection Czechoslov. Chem. Commun. *36,* 218 (1971); Jošt K., Šorm F.: Collection Czechoslov- Chem. Commun. *36,* 234 (1971); Rudinger J., Jošt K.: Cs patent 123272 (PV 2429—61); Frič I., Kodíček M., Procházka Z., Jošt K., Bláha K.: Collection Czechoslov. Chem. Commun. *39,* 1290 (1974); Jošt K., Barth T., Krejčí I., Šorm F.: Cs patent 149028 (PV 1122—71)) and were available for experiments as freeze-dried powders. Chemical, physicochemical and pharmacological properties of some examples are listed in Table 1. All of these substances are easily soluble in water and solutions for injection were prepared containing 0.1 mg/ml physiological saline, under sterile conditions, at pH 3 to 4. Such solutions were kept at 5°C under sterile conditions.

Use of these substances has a number of advantages:

1. Both for the health of the newborns and the cost in materials and time in care, it is of advantage to have a long-acting oxytocic effect which can initiate the entire process of labour and birth with a single injection (5 μg/kg).

In the case of calves it is important for their health that they get colostrum, for development of their own immunoglobuline, as soon as possible after birth. This is simpler to guarantee if they are born in the daylight hours of a normal working day, which the active substances used in this invention enable. The chemical basis of the claimed activities are based on a) absence of an N^{α}-amino group of 1-cysteine which hinders aminopeptidase cleavage of the molecule into inactivity, b) replacement of the disulphide bridge by a thioether one, which eliminates disulphide cleavage of the molecule into inactivity and c) if desired, replacement of the p-OH group of 2-tyrosine by a methoxy group. With the prolonged activity thus produced, labour can be induced early in the morning on a working day, and birth can take place in the afternoon of the same day — this several days to one week previous to the date of expected onset of spontaneous labour. In swine, labour is prolonged due to the large litter size and frequently the last piglets to be born are anoxic and either die or the mother kills them. The oxytocin analogue used in the present invention in the same dosage accelerate labour without producing uterine vasoconstriction, and thus increase the percentage of viable, healthy young.

2. In low dosage (1 μg/kg) given as a single injection there is prolonged evacuation of the udder and increased milk delivery. It is known that these analogues have a prolonged contractile effect on the isolated mammary gland of the rat (Barth T., Jošt K., Rychlík I.: Endocrinol. exp. *9,* 35 (1975); Barth T., Flegel M., Jošt K.: Endocrinol. exp. *10,* 65 (1976)) but not until the experience in example 1 (cf. below) was it shown that one injection can produce milk ejection in an unanaesthetised cow for many hrs.

Examples of use of these analogues are given below:

EXAMPLE 1.

Induction of labour and milk ejection in the cow

The substances presented in Table 1 are analogues of oxytocin, in the main with a higher potency (Barth T., Krejčí I., Kupková B., Jošt K.: Europ. J. Pharmacol. *24,* 183 (1973)) and more prolonged action in inducing separate contractions of the rat uterus in vivo (Barth T., Krejčí I., Vaněčková J., Jošt K., Rychlík I.: Europ. J. Pharmacol. *25,* 65 (1974)) than the parent hormone. These experimental conditions did not allow any conclusion as to whether this prolonged activity would also be observed in pregnant animals and whether in unanaesthetised, intact animals there would be useful, phasic contractions of the pregnant uterus which would serve to expel the foetus. For this reason the following was carried out:

A pregnant cow, body wt. 630 kg, on the 287th day of pregnancy — 2 days before the expected onset of labour — was used. The cervix was dilated to 3 cm at the external os,

the internal os was closed. The udder was well filled with colostrum. No spontaneous uterine contractions were observed and the foetus had not started its "descent" into the birth passages. Labour was *NOT* in progress. The cow received three consecutive dosage of analogue Ic (cf. Table 1) into the jugular vein: 0.5, 1.0 and 1.5 mg. Within 5 min after the first injection uterine tone increased and there was spontaneous milk ejection from the udder which lasted for about 6 h after the last injection. Since the udder remained full over this period, clearly new colostrum was being formed at an adequate rate. Also by 5 min after the first injection uterine phasic contractions started. There were foetal movements, the cow assumed a "labour stance" and there were clear clinical signs of labour onset, including lying and use of respiratory muscles to "press down". The contractions lasted at first 2—5 min at intervals of 10—20 min, and then frequency increased. There were no clinical signs of toxicity in terms of respiration or behaviour of the animal. The foetus began its "descent" and there was gradual dilation of the cervix (50 min — external os 7 cm, mucous plug extruded; 60 min — external os 8 cm, internal os 2 cm; 180 min — external os 8 cm, internal os 3 cm; 280 min — external os 10 cm, internal os 5 cm). A healthy calf was born 8 h after a total dose of 3 mg, i.e. about 5 μg/kg body wt. Since separate injections of oxytocin last only 20 min, the long term action of the analogue was clearly demonstrated, with useful phasic contractions. After birth the cow was in good condition and subsequent lactation was good. No signs of toxicity were observed in either the cow or the calf. The course of the induced labour was normal. The entire birth process could be evoked by a single injection i.v. near to the normal term date of the animal.

Similar experience was had with a pregnant sow which delivered 12 healthy piglets.

In addition to the detailed course of induced labour in a single cow illustrated above, labour has been induced in a total of 6 cows: in 4 the only application was 5 mg of the claimed substance i.v. once only. In two this same induction was followed by a second injection of 2.5 mg per cow. The mean internal (± SEM) between first induction and final delivery was 4.7 hours ± 1.7, with a range from 0.5 to 8.5 hours, i.e. well within a single work shift. In all cases, no signs of toxicity were observed, labour contractions were organised and effective, healthy, normal, non-anoxic calves were delivered, with no signs of metabolic acidosis and they fared well after birth. The cows were normal and lactated well.

EXAMPLE 2.

Facilitation of conception. Action on the oviducts

Oxytocin has a short-lived peristaltic, contractile action on the oviducts. This activity clearly accelerates motion of ovulated eggs towards the uterine cavity where, if fertilised, nidation in the endometrium can occur provided that uterine haemodynamics are not interfered with.

White female mice, body wt. 20 g, were followed by vaginal smear to determine the phase of their oestrus cycle. Mice in oestrus were anaesthetised with pentobarbital (30 mg/kg or about 0.6 mg/mouse, i.p.) the abdominal cavity was opened anteriorly and the oviducts with visible egg clusters were observed under a dissecting microscope. Under conditions of anaesthesia, no peristaltic movements could be observed. 2 ng/mouse of oxytocin were injected i.v. and in 10 animals peristaltic activity induced in the oviducts lasted for 22.6 ± 3.5 min. A dose of 2 ng/mouse of analogue Ib (cf. Table I) i.v. induced contractions lasting 143 ± 15 min. Analogue Ic (cf. Table 1) showed the same response duration as analogue Ib, but at a dose of 20 ng/mouse. The same experiments were repeated with the same result when ovulation in the mouse was induced by injection of 20 units of pregnant mare serum gonadotrophin (experiments were carried out 18 h after gonadotrophin injection).

In cattle artificial insemination is carried out with frozen sperm, which has lost some of its capacity. The frequency of impregnation after a single insemination in cows is about 50%. An increase in this percentage would have considerable economic importance (Knifton A.: Res. vet. Sci. *18,* 288 (1975)). If we shorten the time interval necessary for contact of sperm with eggs, the probability of conception should increase (Guiloff E., Ibarra-Polo A.A., Gomez-Rogers C.: Fertil. Steril. *25,* 946 (1974); Hauschild R., Seewald H.J.: Zenbl. Gynäk. *96,* 1030 (1974); Jones D.E., Ruchesbusch Y., Bayard F.: J. Reprod. Fert. *43,* 23 (1975)) particularly if we use an analogue under the present claims which, in the dosage used, does not produce endometrial vasoconstriction. Since sperm-egg contact can occur several h after insemination, it is clear that the prolonged action of the above analogue is of advantage because of the long duration of the oviductal peristaltis. The application would i.v., 1 μg/kg simultaneous with insemination.

In human medicine there are cases in which labour is either prolonged and ineffective or induced labour is indicated (i.e. diabetic mothers with large foetuses). Here again, the above analogues, in i.v. dosages of 200—300 μg/kg, can be of use.

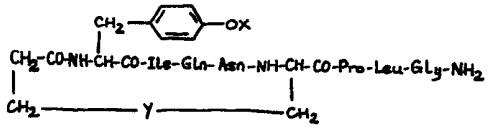
TABLE I

Substance	Formula (m.w.)	Analysis %C	%H	%N (calculated/found)	Optical rotation (solvent)	K	R_f TLC SBN/SBA	Biological activities (IU/mg) A	B	C	D	E	F
Ia	$C_{44}H_{67}N_{11}O_{12}$	50.52	7.23	14.73	–71.7°	2.15	0.20	1899	1206	1127	581	604	24
X = H, Y = CH_2S	$S.4H_2O$ (1046)	50.42	6.62	14.61	(water)		0.35						
Ib	$C_{44}H_{67}N_{11}O_{12}$	53.26	7.01	15.53	–84.5°	1.95	0.20	922	2792	571	755	456	118
X = H, Y = SCH_2	$S.H_2O$ (992)	53.13	7.09	14.90	(1 M CH_3COOH)		0.35						
Ic	$C_{45}H_{69}N_{11}O_{12}$	52.78	7.18	15.05	–69.0°	3.15	0.35	10	40	–	67	35	9.0
X = CH_3, Y = CH_2S	$S.2H_2O$ (1024)	52.29	6.90	14.88	(1 M CH_3COOH)		0.36						

K = partition coefficient between 2-butanol and 0.05% acetic acid; SBN = 2 butanol, 25% ammonia, water (85:7.7:7.5); SBA = 2 butanol, 90% formic acid, water (75:13.5:11.5). A = isolated rat uterus, B = in situ rat uterus, C= vasodepressor in the cock, D = isolated rat mammary gland, E = lactational activity in vivo (rat), F = antidiuretic in the rat. TLC = thin-layer chromatography.

WHAT WE CLAIM IS:—

1. A process for the induction of labour, facilitation of fertilisation and/or increasing milk production in non-human mammals, comprising administering a compound of the general formula I:



where X represents H or CH_3 and Y represents $—CH_2S—$ or $—SCH_2—$, the administration of the compound being by injection.

2. A process as claimed in Claim 1, wherein the drug is applied at a dosage within the range of 10 ng. to 100 μg/kg. body weight.

3. A process as claimed in Claim 1 or 2, wherein the mammal is a farm animal.

4. A process as claimed in Claims 1, 2 or 3, substantially as hereinbefore described.

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