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(57) Abstract

The present invention relates to the use of substances with oxytocin activity in order to create well-being. It also relates to a pharmaceutical composition comprising at least one substance with oxytocin activity in order to create well-being.

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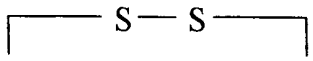
USE OF SUBSTANCES WITH OXYTOCIN ACTIVITY IN ORDER TO CREATE WELL-BEING

The present invention relates to the use of substances with oxytocin activity in order to create well-being. It also relates to a pharmaceutical composition comprising at least one substance with oxytocin activity in order to create well-being.

Background of the invention

Oxytocin was one of the first peptide hormones to be isolated and sequenced. It is a nonapeptide with two cysteine residues that form a disulfide bridge between positions 1 and 6 and corresponds to the formula



For a long time the only effects attributed to oxytocin were its stimulating effects on milk ejection and uterine contractions, but in the past decades it has been shown that oxytocin exerts a wide spectrum of effects within the central nervous system, CNS. It has been suggested that oxytocin participates in the control of memory and learning processes and of various types of behaviour such as feeding, locomotion, as well as maternal and sexual behaviour. Oxytocin is also suggested to participate in the control of cardiovascular functions, thermoregulation, pain threshold and fluid balance. There is also evidence that oxytocin is involved in the control of various immunological processes. It has recently been demonstrated that oxytocin injections cause a lowering of blood pressure and increased weight gain - long lasting effects after repetitive administration. As a central stimulating substance oxytocin plays an important role in the interaction between mother and progeny in mammals. The products may also be used prophylactic in young human beings e.g. already in new born babies or young children to prevent the development of diseases later on in life

which diseases are dependent on stress conditions during the fetal life. Such conditions may be heart/vessel diseases such as stroke, heart infarct, hypertension, and diabetes.

There are different processes described for the synthetical production of oxytocin; commercial processes are for instance described in US patents 2,938,891 and 3,076,797.

In the human body oxytocin is produced in the paraventricular nucleus, PVN, and the supraoptic nucleus, SON, of the hypothalamus. It differs by only two amino acids from vasopressin, which is also produced in these nuclei. The magnocellular oxytocinergic neurons of the SON and PVN send Parvocellular neurons that originate in the PVN project into multiple areas within CNS. The oxytocin-producing cells are innervated by cholinergic, catecholaminergic as well as peptidergic neurons. The presence of oxytocin in different tissues outside the brain, such as the uterus, ovaries, testis, thymus, adrenal medulla and pancreas has been demonstrated and oxytocin is suggested to exert local effects in these organs.

A parallel secretion of oxytocin into the brain regions and into the circulation occurs in response to some stimuli such as suckling, but other stimuli can cause separate activation of oxytocinergic neurons, terminating in the brain or the pituitary.

In this context oxytocin refers, whenever applicable, in addition to oxytocin also to precursors, metabolic derivatives, oxytocin agonists or analogues displaying the same properties.

There are several oxytocin derivatives, i.e. compounds with a structure similar to that of oxytocin. There are preliminary indications that other oxytocin derivatives than oxytocin could give the well-being effects of oxytocin as well as parts of the oxytocin molecule. No publications describe the use of other oxytocin derivatives than oxytocin or parts of the oxytocin molecule to create well-being.

In experiments it has been shown that oxytocin by way of a central action increases the activity of the central α_2-receptors in rats. These receptors have an inhibitory action and counteracts the activating aspects of noradrenalin in the brain which are mainly mediated via α_1-receptors, which activate cyclic AMP. When α_2-receptor stimulation dominates over α_1-receptor stimulation, activity is exchanged by relaxation and energy is shunted towards growing and healing, i.e. is not used for stress or muscular contraction and activity. As a consequence parasympathetic nerve tone dominates over sympathetic nervous tone and the musculature is relaxed. It can be presumed that oxytocin exerts a similar effect also in humans. During breast feeding - a situation characterized by repetitive oxytocin secretion - all the effects observed in experimental animals following repeated oxytocin administration are seen. It is not known how the effect by oxytocin on α_2-receptors is mediated, but probably not by a classical oxytocin receptor mediated effect.

The effect of oxytocin can be extended or strengthened by administration in combination with drugs increasing the release of oxytocin and/or the number or affinity of receptors, such as estrogen, or drugs having an α_2-agonistic effect, such as clonidine.

Uvnäs-Moberg, K., Ahlenius S., Hillegaart, V., and Alster P., High Doses of Oxytocin Cause Sedation and Low Doses Cause an Anxiolytic-Like Effect in Male Rats, Pharmacology Biochemistry and Behaviour, Vol. 49, No. 1, pp. 101-106, 1994, showed that treatment of male rats with 250-1000 µg/kg of oxytocin caused sedative effects as indicated by a suppression of locomotor activity and rearing. Uvnäs-Moberg, K., Alster, P., and Petersson, M., Dissociation of Oxytocin Effects on Body Weight in Two Variants of Female Sprague-Dawley Rats, Integrative Physiological and Behavioral Science, Vol. 31, No. 1, pp. 44-55, January-March 1996, showed that oxytocin 1 mg/kg given subcutaneously to female Sprague-Dawley Rats for a five-day period increased weight gain significantly in comparison to a NaCl control. Uvnäs-Moberg, K., Antistress Pattern Induced by Oxytocin, News Physiol. Sci., Vol. 13, pp. 22-26, February 1988, showed that repeated oxytocin injections to rats

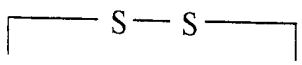
caused lowered blood pressure, decreased cortisol levels, increased withdrawal latency, increased release of vagally controlled gastrointestinal hormones, and increased weight gain. Together, these effects form an antistress pattern.

Summary of the invention

The present invention relates to the use of a substance with oxytocin activity in order to create well-being. The invention also relates to a pharmaceutical composition comprising an effective concentration of at least one substance with oxytocin activity in mixture or otherwise together with at least one pharmaceutically acceptable carrier or excipient. Such a pharmaceutical composition could be used in order to create well-being.

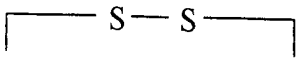
Detailed description of the invention

An object of the present invention is the use of a substance with an oxytocin like activity in order to create well-being. Examples of substances with oxytocin activity are the following compounds:



Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2

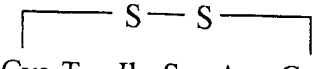
Oxytocin



Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Ile-Gly-NH_2

Mesotocin

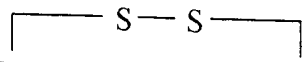
i.e. V is Tyr, W is Ile, X is Gln, Y is Ile, and Z is Gly in Claim 2 and 4



Cys-Tyr-Ile-Ser-Asn-Cys-Pro-Ile-Gly-NH_2

Isotocin

i.e. V is Tyr, W is Ile, X is Ser, Y is Ile, and Z is Gly in Claim 2 and 4



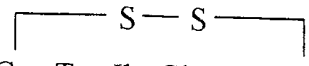
Cys-Phe-Val-Arg-Asn-Cys-Pro-Thr-Gly-NH_2

Annetocin

i.e. V is Phe, W is Val, X is Arg, Y is Thr, and Z is Gly in Claim 2 and 4

Annetocin has been isolated from the earthworm, as described in Oumi T, Ukena K, Matsushima O, Ikeda T, Fujita T, Minakata H, Nomoto K, Annetocin: an oxytocin-related peptide isolated from the earthworm, Eisenia foetida, Biochem Biophys Res Commun 1994, Jan 14; 198(1): 393-399. Other substances with oxytocin activity could also be used, such as naturally occurring or artificially modified variants, analogues, and derivatives of oxytocin, mesotocin, isotocin, and annetocin. Such substances could be obtained by addition, insertion, elimination, or substitution of at least one amino acid in these hormones. By substance with an oxytocin like activity is also understood precursors, metabolites such as metabolic derivatives e.g. metabolic degradation products, agonists, or analogues of the substances mentioned herein displaying the same properties. Metabolic derivatives or metabolic degradation products may be oxytocin like peptides e.g. with nine amino acids such as oxytocin, mesotocin, isotocin, and annetocin from which one or more amino acids has been deleted from either or both ends of the molecule. Preferably one, two or three amino acids may have been deleted from the carboxy terminal end i.e. Gly only, Gly and Leu, or Gly, Leu, and Pro. Preferably one, two or three amino acids may have been deleted from the amino terminal end i.e. Cys only, Cys and Tyr, or Cys, Tyr, and Ile. Preferably, one, two or three amino acids may have been deleted both from the carboxy terminal end i.e. Gly only, Gly and Leu, or Gly, Leu, and Pro, and one, two or three amino acids may have been deleted from the amino terminal end i.e. Cys only, Cys and Tyr, or Cys, Tyr, and Ile. It could be ascertained that these variants are analogues of oxytocin, mesotocin, isotocin or annetocin by immunological methods, e.g. RIA (radio-immunoassay), IRMA (radiometric methods), RIST (radioimmunosorbent test), RAST (radioallergosorbent test).

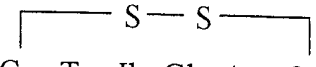
As mentioned above there are indications that subfragments of the oxytocin molecule could give well-being. Preferably, these subfragments of the oxytocin molecule is made by deletions of amino acids outside the disulfide bridge. Examples of subfragments of the oxytocin molecule are the following compounds:



Cys-Tyr-Ile-Gln-Asn-Cys-Pro-NH_2

SEQ ID NO: 1

i.e. V is Tyr, W is Ile, X is Gln, Y is nothing, and Z is nothing in Claim 2 and 4

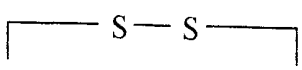


Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-NH_2

SEQ ID NO: 2

i.e. V is Tyr, W is Ile, X is Gln, Y is Leu, and Z is nothing in Claim 2 and 4

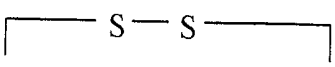
There is also a possibility to create new compounds with oxytocin activity by means of computer simulation. Methods for computer simulation are known by a person skilled in the art, e.g. as described in EP 0660 210 A2. Seven new compounds have been created by means of computer simulation, namely the following peptides:



Cys-Tyr-Val-Thr-Asn-Cys-Pro-Leu-Gly-NH_2

SEQ ID NO: 3

i.e. V is Tyr, W is Val, X is Thr, Y is Leu, and Z is Gly in Claim 2 and 4



Cys-Tyr-Hoph-Thr-Asn-Cys-Pro-Val-Gly-NH_2

SEQ ID NO: 4

i.e. V is Tyr, W is Hoph, X is Thr, Y is Val, and Z is Gly in Claim 2 and 4

S S

Cys-Tyr-Phe-Cit-Asn-Cys-Pro-Leu-Gly-NH_2

SEQ ID NO: 5

i.e. V is Tyr, W is Phe, X is Cit, Y is Leu, and Z is Gly in Claim 2 and 4

S — S

Cys-Tyr-Cha-Arg-Asn-Cys-Pro-Hos-Ala-NH_2

SEQ ID NO: 6

i.e. V is Tyr, W is Cha, X is Arg, Y is Hos, and Z is Ala in Claim 2 and 4

S — S

Cys-Tyr-Val-Daba-Asn-Cys-Pro-Daba-Ala-NH_2

SEQ ID NO: 7

i.e. V is Tyr, W is Val, X is Daba, Y is Daba, and Z is Ala in Claim 2 and 4

S — S

Cys-Tyr-Hoph-Daba-Asn-Cys-Pro-Cit-Ala-NH_2

SEQ ID NO: 8

i.e. V is Tyr, W is Hoph, X is Daba, Y is Cit, and Z is Ala in Claim 2 and 4

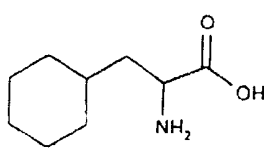
S — S

Cys-Tyr-Phe-Arg-Asn-Cys-Pro-Val-Ala-NH_2

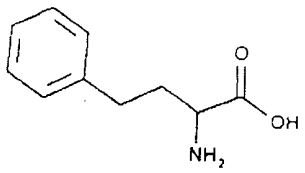
SEQ ID NO: 9

i.e. V is Tyr, W is Phe, X is Arg, Y is Val, and Z is Ala in Claim 2 and 4,

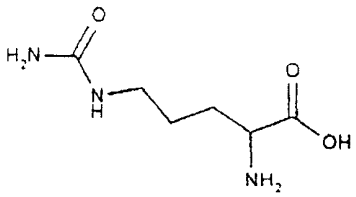
wherein Cha stands for cyclohexylalanine,



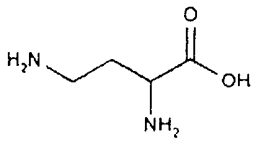
Hoph stands for homophenylalanine,



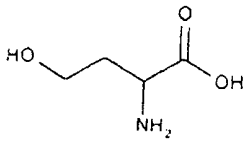
Cit stands for citruline,



Daba stands for diaminobutyric acid, and



Hos stands for homoserine.



The invention also relates to the peptides mentioned above in both D- and L-form. Especially the invention relates to the L-form. By inversion of the peptide sequence thereof, the D-form could be converted to the L-form. The effect of the D- and L-forms are the same. These and the peptides above can be produced by methods

known to a person skilled in the art, e.g. according to Merrifield, P.B., "Solid Phase Synthesis", *Angew. Chemie*, **1985**, No. 97, p. 801.

It could be ascertained that those compounds prepared by computer simulation have oxytocin effect by tests, such as described in the publications by Uvnäs-Moberg, K. above.

By the expression "well-being" we understand a balanced condition resulting in increased concentration and receptivity, which lead to increased learning capacity and increased efficiency. Other results from this balanced condition are calmness, relaxation, sedative effect, nourishment takeup, growth, and antistress effect.

Another object of the invention is a pharmaceutical composition comprising an effective concentration of at least one substance with oxytocin activity in mixture or otherwise together with at least one pharmaceutically acceptable carrier or excipient. Examples of substances with oxytocin activity are mesotocin, isotocin, annetocin, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.

The pharmaceutical compositions are prepared in a manner known to a person skilled in the pharmaceutical art. The carrier or the excipient could be a solid, semi-solid or liquid material that could serve as a vehicle or medium for the active ingredient. Suitable carriers or excipients are known in the art. The pharmaceutical composition could be adapted to oral or parenteral use and could be administered to the patient as tablets, capsules, suppositories, solutions, suspensions or the like.

The pharmaceutical compositions could be administered orally, e.g. with an inert diluent or with an edible carrier. They could be enclosed in gelatin capsules or be compressed to tablets. For oral therapeutic administration the compounds according to the invention could be incorporated with excipients and used as tablets, lozenges, capsules, elixirs, suspensions, syrups, wafers, chewing gums and the like. These

preparations should contain at least 4% by weight of the compounds according to the invention, the active ingredient, but could be varied according to the special form and could, suitably, be 4-70% by weight of the unit. The amount of the active ingredient that is contained in compositions is so high that a unit dosage form suitable for administration is obtained.

The tablets, pills, capsules, lozenges and the like could also contain at least one of the following adjuvants: binders such as microcrystalline cellulose, gum tragacanth or gelatin, excipients such as starch or lactose, disintegrating agents such as alginic acid, Primogel, corn starch, and the like, lubricants such as magnesium stearate or Sterotex, glidants such as colloidal silica dioxide, and sweetening agents such as saccharose or saccharin could be added or flavourings such as peppermint, methyl salicylate or orange flavouring. When the unit dosage form is a capsule it could contain in addition of the type above a liquid carrier such as polyethylene glycol or a fatty oil. Other unit dosage forms could contain other different materials that modify the physical form of the unit dosage form, e.g. as coatings. Accordingly, tablets or pills could be coated with sugar, shellac or other enteric coating agents. A syrup could in addition to the active ingredient contain saccharose as a sweetening agent and some preservatives, dyes and flavouring agents. Materials that are used for preparation of these different compositions should be pharmaceutically pure and non-toxic in the amounts used.

For parenteral administration the compounds according to the invention could be incorporated in a solution or suspension. Parenteral administration refers to the administration not through the alimentary canal but rather by injection through some other rute, as subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, intravenous, intranasal, intrapulmonary, through the urinary tract, through eye drops, rectal or vaginal (e.g. as a suppository, a vagitorium, a cream or an ointment), through the lactiferous tract in cattles, into an organ such as bone marrow, etc. Bone marrow may also be treated *in vitro*. These preparations could contain at least 0.1% by weight of an active compound according to the invention but could be

varied to be approximately 0.1-50% thereof by weight. The amount of the active ingredient that is contained in such compositions is so high that a suitable dosage is obtained.

The solutions or suspensions could also comprise at least one of the following adjuvants: sterile diluents such as water for injection, saline, fixed oils, polyethylene glycols, glycerol, propylene glycol or other synthetic solvents, antibacterial agents such as benzyl alcohol or methyl paraben, antioxidants such as ascorbic acid or sodium bisulfite, chelating agents such as ethylene diamine tetraacetic acid, buffers such as acetates, citrates or phosphates, and agents for adjustment of the tonicity such as sodium chloride or dextrose. The parenteral preparation could be enclosed in ampoules, disposable syringes or multiple dosage vessels made of glass or plastic.

For topical administration the compounds according to the invention could be incorporated in a solution, suspension, or ointment. These preparations could contain at least 0.1% by weight of an active compound according to the invention but could be varied to be approximately 0.1-50% thereof by weight. The amount of the active ingredient that is contained in such compositions is so high that a suitable dosage is obtained. The administration could be facilitated by applying touch, pressure, massage or heat, warms, or infrared light on the skin, which leads to enhanced skin permeability. Hirvonen, J., Kalia, YN, and Guy, RH. Transdermal delivery of peptides by iontophoresis, *Nat Biotechnol* 1996 Dec; **14(13)**: 1710-1713 describes how to enhance the transport of a drug via the skin using the driving force of an applied electric field. Preferably, iontophoresis is effected at a slightly basic pH.

Other administration forms are inhalation through the lungs, buccal administration via the mouth and enteral administration via the small intestine that could be effected by means known by a person skilled in the art.

All publications mentioned herein are hereby incorporated by reference. By the expression "comprising" we understand including but not limited to. Thus, other non-mentioned substances, additives or carriers may be present.

Short description of the Figures

Figure 1. Experimental design. The study was divided into two experiments (I and II). The time-course for the different behavioral tests and decapitation is shown in the figure. In the two experiments, 5 rats from each of the two Sprague-Dawley substrains A and B were tested.

Figure 2. Acquisition of a conditioned avoidance response in two substrains of Sprague-Dawley rats. The animals were tested in 5 daily sessions of 10 min duration each. Shown are the medians ± semiinterquartile range, based on observations of 10 rats per group (cf. Fig. 1). Statistical analysis was performed by means of a Mann-Whitney U-test (see Siegel, J., Sisson, D.F., Driscoll, P. (1993). Augmenting and reducing of visual evoked potentials in Roman high- and low-avoidance rats. Physiol Behav 54, 707-711) at the respective days, as shown in the figure.
ns $p>0.05$ **$p<0.01$

Figure 3. Startle amplitude and response latency in two substrains of Sprague-Dawley rats. Statistical description and analysis, as in legend to Fig. 2.
** $p<0.01$

Figure 4. Spontaneous locomotor activity in two substrains of Sprague-Dawley rats. The rats were tested for 15 days in the open-field arena. Statistical description and analysis as in legend to Fig. 2.
ns $p>0.05$

Figure 5. Visual analogous scale. The correlationship between oxytocin concentration ($pmolL^{-1}$ in the blood) and happiness in a group of stressed women. The happi-

ness is scored from 0 to 7. The number 0 denotes the least possible happiness and number 7 the most possible happiness.

$r = 0.35$; $p = 0.03$.

The invention will be illuminated by the following Examples, which are only intended to illuminate and not restrict the invention in any way.

Examples

Materials and methods

Animals

Adult male Sprague-Dawley rats (280-320 g) were obtained from two different colonies within the breeding facility (B&K Universal AB, Sollentuna, Sweden). The two colonies, raised in different buildings, are hereafter referred to as Substrain A and Substrain B. The animals arrived in the laboratory at least 10 days before being used in experiments, and were house, five per cage (Makrolon® IV), under controlled conditions of temperature (21.0±0.4°C), relative humidity (55-65%) and light-dark cycle (12:12h, lights off 06.00h). Food (R36, Ewos, Södertälje) and tap water were available ad libitum in the home cage. The animals were provided with ten new cage-bedding and fresh water three times per week (Mon, Wed, Fri). The two sets of animals used in the present study were born on the 18-24th of December 1995 (Experiment I) and on the 12th of January 1996 (Experiment II) (cf. Fig. 1).

The Studies were approved by the Stockholm South Local Ethical Committee on Animal Experiments.

Example 1 - Conditioned avoidance behaviour

The rats were trained to perform a two-way conditioned avoidance response (CAR) in a set of four symmetrical shuttle boxes (Kungsbacka Mät- & Reglerteknik AB, Kungsbacka, Sweden). Each chamber (600 x 310 x 250 mm) was made of perspex and divided into two equal compartments by a partition with an opening (80 x 110 mm). The position of the rat was automatically registered by means of 2 x 4 photocells mounted on the long sides. The floor in the chambers was made up of grids connected to a high-resistance power supply (730 V), resulting in a current of ≤0.6 mA. Upon presentation of the conditioned stimulus [white noise, 70 dB(A)], the rat was given 10 s to avoid an intermittent grid shock (inter-shock interval 2.5 s, shock duration 0.5 s) by moving into the safe compartment. The shuttle-boxes were operated automatically, and the inter-trial interval (end of trial to presentation of white noise in the following trial) varied at random between 20 and 40s, with a session length of 15 minutes. The shuttle boxes were contained in ventilated, sound-attenuating enclosures. For further details, including a schematic drawing of the equipment, see Salmi, P., Samuelsson, J., and Ahlenius, S. (1994). A new computer-assisted two-way avoidance conditioning equipment for rats: Behavioural and pharmacological validation. J Pharmacol Toxicol Meth 32, 155-159.

Example 2 - Motor activity observations

The spontaneous motor activity was observed in a square open-field arena (680 x 680 x 450 mm), equipped with two rows of photocells (8 x 8), sensitive to infrared light. Two identical frames of photocells were placed at two levels, 40 and 125 mm above the floor, respectively. The photocells were spaced 90 mm apart, and the last photocell in a row was spaced 25 mm from the wall. The open-field was enclosed in a ventilated, sound-attenuating box with a perspex top. Measurements were made in the dark and performed between 0.900-16.00 h.

The number of photocell beam interruptions were collected on an IBM-compatible PC computer allowing the registration of locomotor activity (all interruptions of photobeams at the lower level) and rearing (all interruptions of photobeams at the upper level). The data were subject to a square root transformation. For further details on the apparatus and the computer software used, including a schematic drawing of the equipment, see Ericson, E., Samuelsson, J., and Ahlenius, S. (1991). Photocell measurements of rat motor activity: A contribution to sensitivity and variation in behavioral observations. J Pharmacol Meth 25, 111-122.

Example 3 - Startle response equipment

For startle response amplitude, and latency to the startle response, commercially available equipment was used (Metod & Produkt, Svenska AB, Göteborg, Sweden). Briefly, the rat was placed in a stainless steel wire-mesh cage (185 x 70 x 65 mm), suspended at one point to a piston in such a way that the cage could freely move under the piston. A sudden movement of the rat inside the cage caused a displacement of the piston, the force of which was converted to an analogue signal by a moving coil transducer. Startle amplitude was defined as the maximum signal amplitude that occurred during the first 40 ms after delivery of the startle-eliciting stimulus. In addition, the latency to the startle response, thus defined, was recorded. The equipment was contained in a sound-attenuating enclosure (520 x 420 x 380 mm) with a masking background noise of 62 dB(A). The startle stimulus tone was 105 dB(A). The animals were subjected to a series of 10 startle stimulus presentations, separated by 10 s. For each rat the mean startle amplitude, and reaction time was calculated. For further details see Johansson, C., Jackson, D.M., Zhang, J., and Svensson, L. (1995). Prepulse inhibition of acoustic startle, a measure of sensorimotor gating. Effects of antipsychotics and other agents in rats. Pharmacol Biochem Behav 52, 649-654.

Example 4 - Oxytocin determination

Oxytocin was measured with a specific radioimmunoassay developed in this laboratory (Stock, S., and Uvnäs-Moberg, K. (1988). Increased plasma levels of oxytocin in response to stimulation of the sciatic and vagal nerves and in response to touch and pinch in anaesthetized rats. Acta Physiol Scand 132, 29-34.). Plasma samples were purified with reversed-phase chromatography using C_{18} Waters SEP-PAK cartridges. The antiserum anti-oxytocin (rabbit) for RIA, KA-19 (Euro Diagnostica, Malmö, Sweden) and the tracer [^{125}I]-Tyr^2-oxytocin (Du Pont NEN Research Products, Boston, MA) were used. The cross-reactivity of the antibody was less than 0.01% with vasopressin, somatostatin LH-RH and ACTH. The limit of detection was 2 pmol L^{-1}. The intra- and interassay coefficients of variation were 11 and 13%, respectively.

Sample collection

Trunk blood (5-7 mL) was collected in ice-chilled tubes, containing heparin (10 IU mL^{-1}) (Pharmacia-Upjohn, Stockholm, Sweden) and aprotenin (500 IU mL^{-1}) (Trasylol®, Bayer, Leverkusen, Germany), and was centrifuged for separation of plasma. Blood sampling, in relation to the various behavioral tests are shown in Fig. 1.

Statistical procedures

Non-parametric statistical description and analysis was used for presentation of behavioral and biochemical results in Fig:s 2-4, as detailed in the description of the Figures.

Results

Behavioral Observations

Conditioned avoidance acquisition: Substrain A displayed a rapid, and statistically significant, acquisition of the avoidance behavior [$\chi^2(4)=27.58$, $p<0.01$, Friedman two-way ANOVA]. In stark contrast, the animals of Substrain B did not improve their performace within the 5 days daily training sessions [$\chi^2(4)=0.25$, n.s., Friedman two-way ANOVA]. In addition, except for Day 1, there was a marked and statistically significant difference between the two groups over the daily training sessions (Fig. 2).

Startle behavior: The Substrain B animals displayed a much stronger response to the auditory startle stimulus, in comparison with Substrain A. Furthermore, the reaction time was statistically significantly shorter for the Substrain B, than for Substrain A, animals (Fig. 3).

Spontaneous motor activity: All animals displayed a high degree of exploratory activity in the open-field arena, and there were no differences in locomotor activity, or rearing behavior, between the two substrains of Sprague-Dawley rats (Fig. 4).

Oxytocin levels: There is a linear correlation between the oxytocin level in the blood and happiness in a group of stressed women (Fig. 5).

Discussion

In the present study, a clear and significant correlation between oxytocin plasma levels and the avoidance performance was found over the Substrain A and B animals. This observation, is of interest in regard to anxiolytic-like properties of oxytocin previously reported from this laboratory (Uvnäs-Moberg, K., Alster, P., Hillegaart, V., and Ahlenius, S. (1992). Oxytocin induces anxiolytic effects and reduces loco-

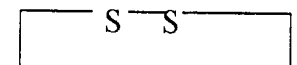
motion in male rats. Acta Physiol Scand 145, 429-430; Uvnäs-Moberg, K., Lundeberg, T., Bruzelius, G., and Alster, P. (1992) Vagally mediated release of gastrin and cholecystokinin following sensory stimulation. Acta Physiol Scand 146). As mentioned above, the high- and low-avoidance performing animals in the Syracuse and Roman lines display very different emotional reactivity. Thus, the low-performing animals from these breeding lines are superior in passive tasks of learning such as the acquisition of a conditioned emotional response and passive avoidance (Brush, F.R., Del Paine, S.N., Pellegrino, I.J., Rykaszewski, I.M., Dess, N.K., and Collins, P.Y. (1988). CER suppression, passive-avoidance learning, and stress-induced suppression of drinkning in the Syracuse high- and low-avoidance strains of rats (Rattus norvegicus). J. Comp Psychol 102, 337-339.) and these animals also display an enhanced stress-induced analgesia (Brush, F.R., and Nagase, C.S. (1989). Endogenous opioids and behavior. In F.R. Brush, and S. Levine (Eds.), Psychoendochrinology, pp. Academic Press, San Diego). Furthermore, the neophobia displayed by low-performing Syracuse animals was shown to be ameliorated by the administration of a benzodiazepine anxiolytic (Flaherty, C.F., and Rowan, G.A. (1989). Rats (Rattus norvegicus) selectively bred to differ in avoidance behavior also differ in response to novelty stress, in glycemic conditioning, and in reward contrast. Behav Neural Biol 51, 145-164). Also in the present study, there was evidence for an increased emotionality in the low-performing animals, as evidenced by an enhanced reactivity to an acoustic stimulation, and a shorter startle latency. It has been shown that when oxytocin is given to rat pups it increases their weight gain in puberty resulting in higher body weight (Uvnäs-Moberg K, Alster P, Petersson M, Sohlström A, Björkstrand E. Postnatal oxytocin injections cause sustained weight gain and increased nociceptive thresholds in male and female rats. Ped Research 1998, 43: 1-5). Of special interest here, the Roman high-performing animals have been found to respond with an increased oxytocin release, as compared to their low-performing counterparts (Carter, D.A., and Lightman, S.L. (1987) Oxytocin stress responses are dependent upon emotionality. Psychoneuroendocrinol 12, 219-223). Of potential interest here, differences were recently reported that in oxytocin-like immunoreactivity in the paraventricular nucleus of the hypothalamus (PVN) and the posterior lobe of the pituitary

between high- and low-avoidance performing animals. Thus, the high-performing animals had a lower oxytocin-like immunoreactivity in the PVN and higher in the posterior lobe, in comparison with the low-performing animals, which displayed the opposite pattern (Zhukov, D.A., and Chernigovskaya, E.V. (1995). Oxytocinergic neurosecretory system in genetically selected rats differing in emotionality, a morphometric investigation. Neurosci Behav Physiol 25, 438-441). Taken together, these observations generally support the notion of anxiolytic-like properties of oxytocin, and that such actions may be of importance for adaptive behavior in a situation of mild stress caused by the demands of, for example avoidance conditioning.

Claims

1. Use of a substance with oxytocin activity for the preparation of a pharmaceutical composition in order to create well-being, such as increased learning capacity and increased efficiency.

2. Use according to Claim 1, **characterized** in that the substance is selected from the group consisting of the following compounds:



Cys- V -W-X-Asn-Cys-Pro-Y-Z-NH_2

wherein

V is selected from the group consisting of Tyr and Phe

W is selected from the group consisting of Ile, Val, Hoph, Phe, and Cha,

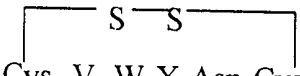
X is selected from the group consisting of Gln, Ser, Thr, Cit, Arg, and Daba,

Y is selected from the group consisting of Ile, Leu, Thr, nothing, Val, Hos, Daba, and Cit,

Z is selected from the group consisting of Gly, nothing, and Ala.

3. Use according to Claim 1-2, **characterized** in that the substance is selected from the group consisting of the following compounds: oxytocin, mesotocin, isotocin, annetocin, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.

4. Pharmaceutical composition in order to create well-being, **characterized** in that it comprises an effective concentration of at least one substance with oxytocin activity in mixture or otherwise together with at least one pharmaceutically acceptable carrier or excipient, wherein the substance is selected from the group consisting of the following compounds:



wherein

V is selected from the group consisting of Tyr and Phe,

W is selected from the group consisting of Ile, Val, Hoph, Phe, and Cha,

X is selected from the group consisting of Gln, Ser, Thr, Cit, Arg, and Daba,

Y is selected from the group consisting of Ile, Leu, Thr, nothing, Val, Hos, Daba, and Cit,

Z is selected from the group consisting of Gly, nothing, and Ala;

with the exception of oxytocin.

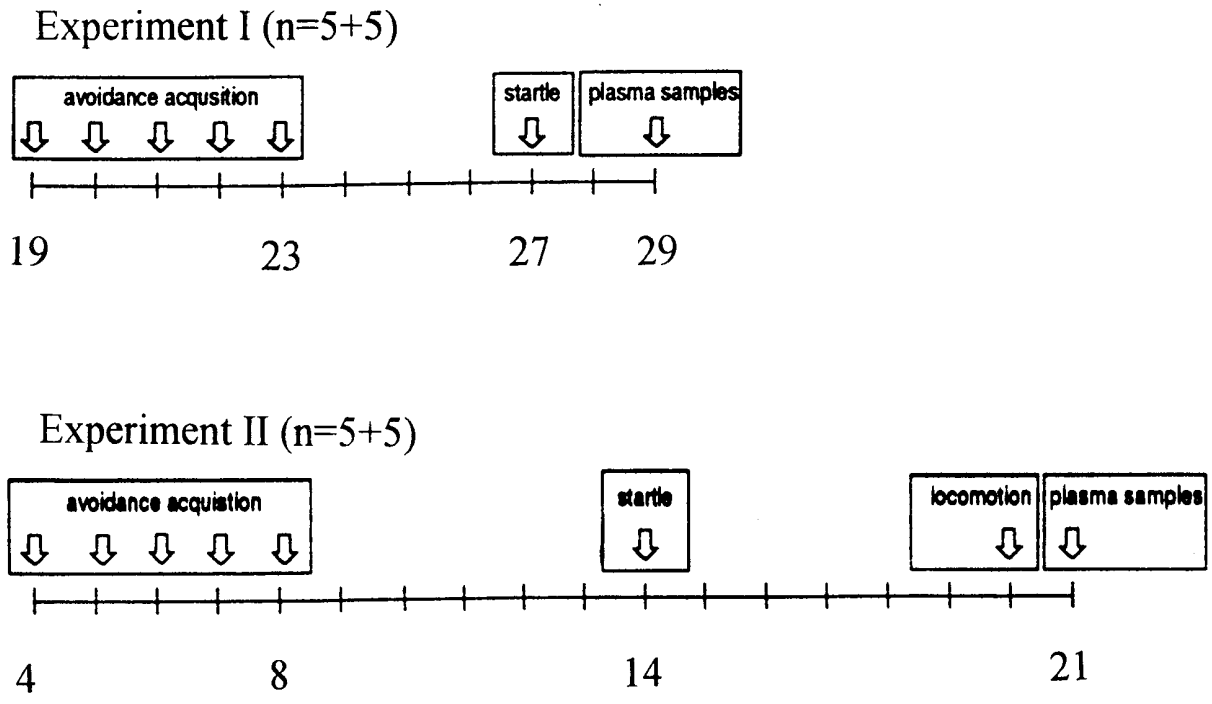
5. Pharmaceutical composition according to Claim 4, **characterized** in that the substance is selected from the group consisting of the following compounds: mesotocin, isotocin, annetocin, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.

6. Pharmaceutical composition according to Claim 4, **characterized** in that the effective concentration is 4-70% by weight.

7. Pharmaceutical composition according to Claim 4 and 6, **characterized** in that the carriers and excipients are selected from: binders such as microcrystalline cellulose, gum tragacanth or gelatin, excipients such as starch or lactose, disintegrating agents such as alginic acid, Primogel, corn starch, and the like, lubricants such as magnesium stearate or Sterotex, glidants such as colloidal silica dioxide, sweetening agents such as saccharose or saccharin, flavourings such as peppermint, methyl salicylate or orange flavouring, liquid carriers such as polyethylene glycol or a fatty oil, coating agents, such as sugar, shellac or other enteric coating agents, preservatives, dyes and flavouring agents.

8. Pharmaceutical composition according to Claim 4, **characterized** in that the effective concentration is 0.1-50% by weight.

9. Pharmaceutical composition according to Claim 4 and 8, **characterized** in that the carriers and excipients are selected from: sterile diluents such as water for injection, saline, fixed oils, polyethylene glycols, glycerol, propylene glycol or other synthetic solvents, antibacterial agents such as benzyl alcohol or methyl paraben, antioxidants such as ascorbic acid or sodium bisulfite, chelating agents such as ethylene diamine tetraacetic acid, buffers such as acetates, citrates or phosphates, and agents for adjustment of the tonicity such as sodium chloride or dextrose.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startle
plasma samples
19
23
27
29
Experiment II (n=5+5)
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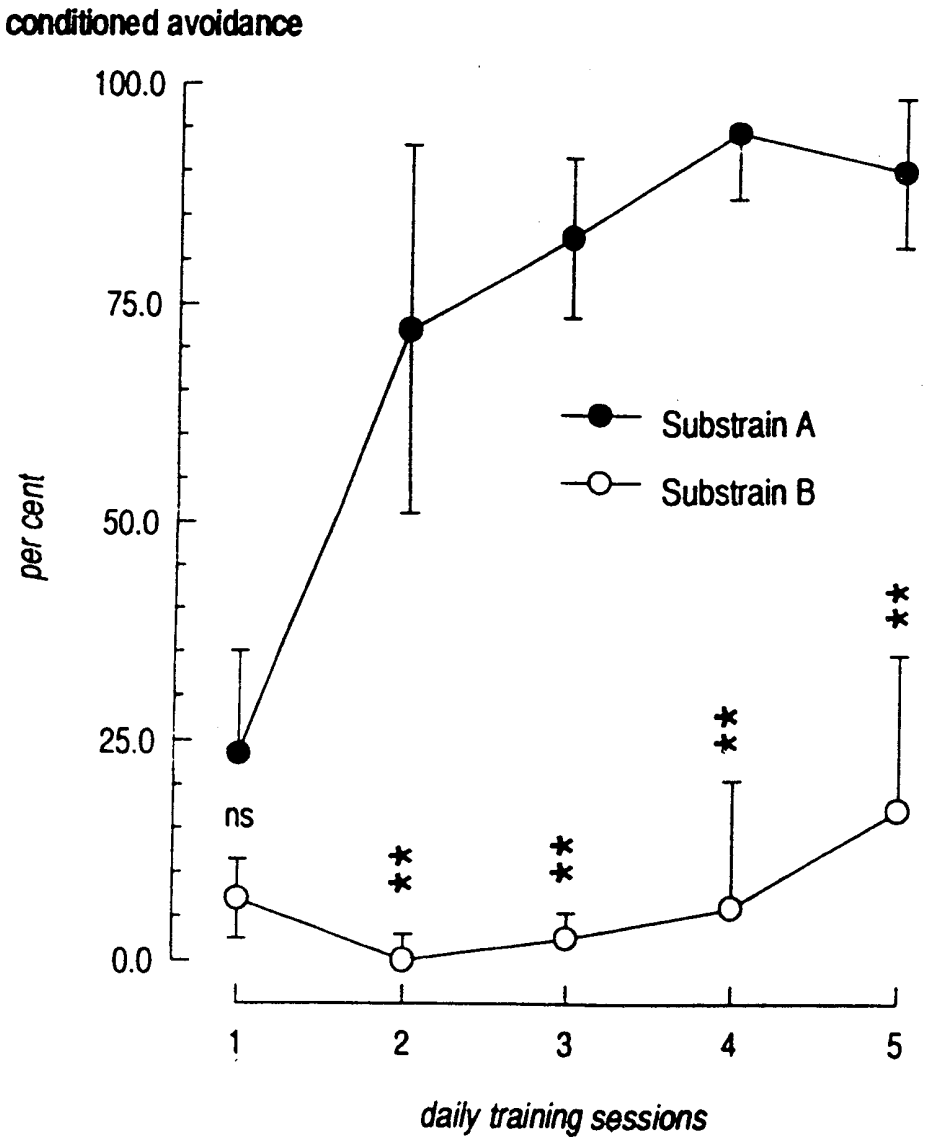
startle
locomotion
plasma samples
4
8
14
21

Fig. 1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per cent
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**
**
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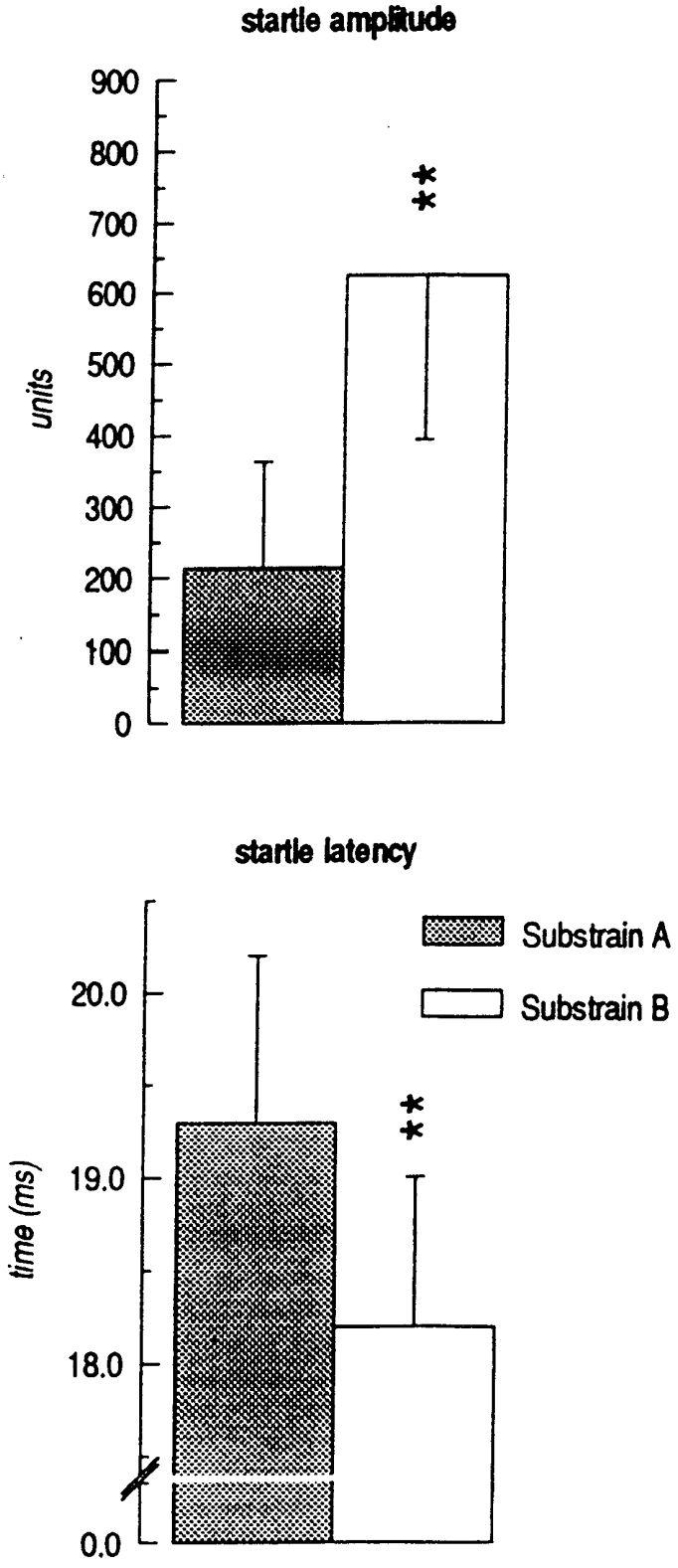
2
3
4
5
daily training sessions

Fig. 2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units
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19.0
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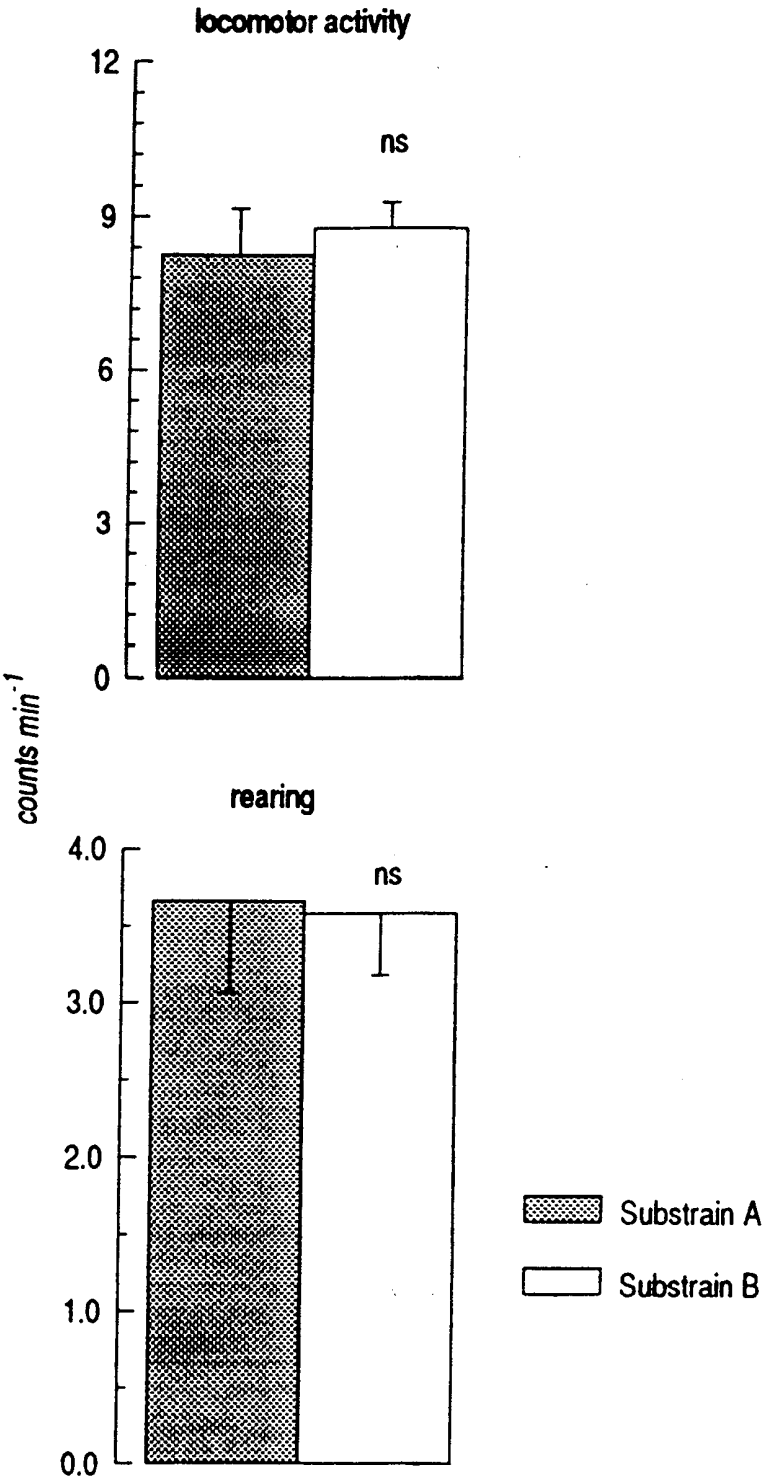
time (ms)
*
*

Fig. 3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Substrain B

Fig. 4

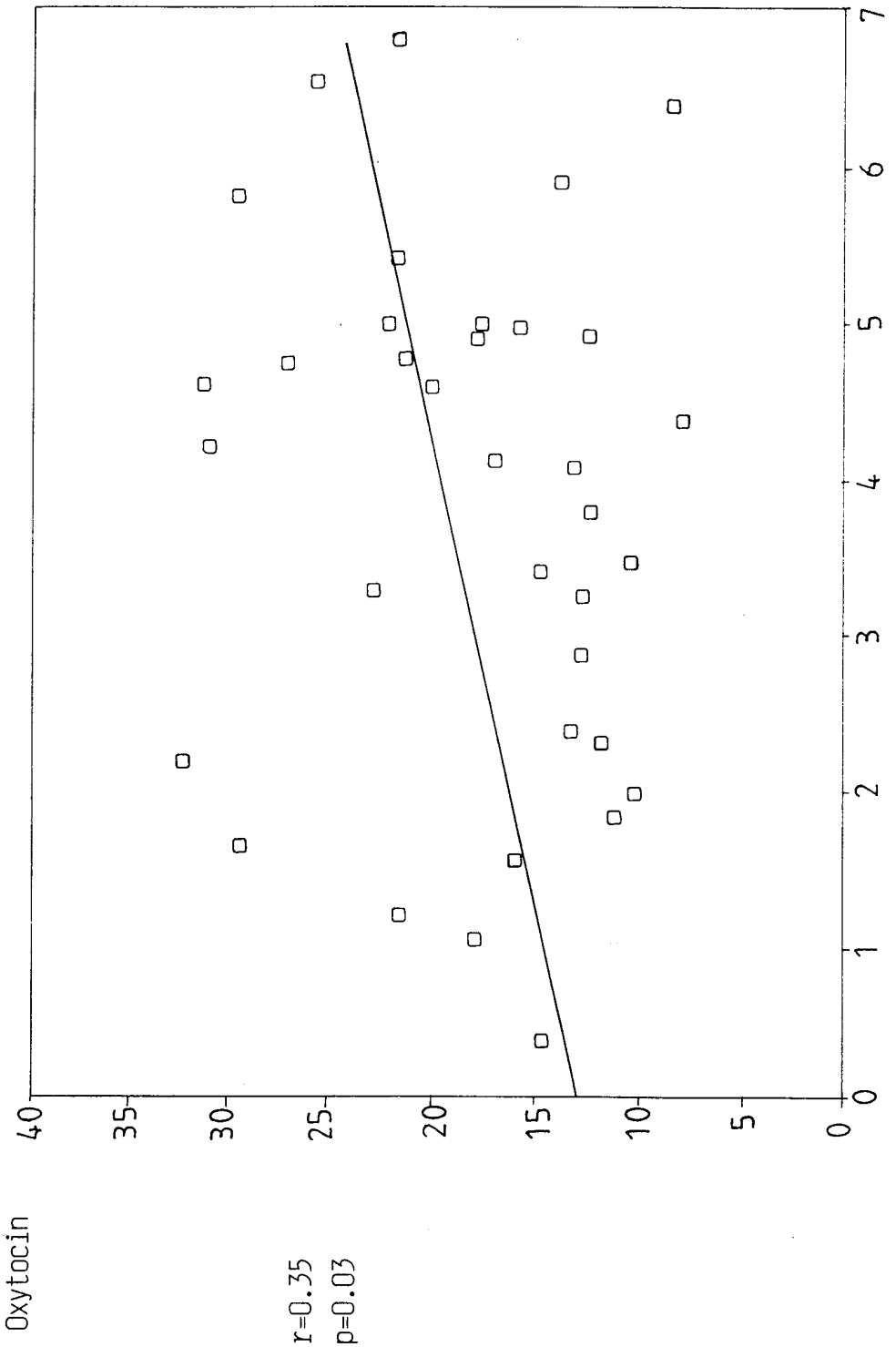

FIG. 5

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01714

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 38/11, A61P 25/24
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REG, WPI, CAPLUS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Psychoneuroendocrinology, Volume 23, No 8, November 1998, Kerstin Uvnäs-Moberg, "Oxytocin May Mediate the Benefits of Positive Social Interaction and Emotions" page 819 - page 835 --	1-9
P,X	Psychopharmacology, Volume 142, 1999, Kerstin Ulvnäs-Moberg, "Oxytocin as a possible mediator of SSRI-induced antidepressant effects" page 95 - page 101 --	1-9
X	Neuropeptides, Volume 29, 1995, R. Arletti et al, "Aged Rats are Still Responsive to the Antidepressant and Memory-impriving Effects of Oxytocin" page 177 - page 182 --	1-9

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 January 2000	31.01.2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carolina Gómez Lagerlöf/Els Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01714

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Acta physiologica Scandinavica, Volume 161, 1997, Kerstin Uvnäs-Moberg, "Oxytocin linked antistress effects - the relaxation and growth response" page 38 - page 42 ---	1-9
X	Neuroscience & Biobehavioral Reviews, Volume 15, 1991, Antonio Argiolas et al, "Central Functions of Oxytocin" page 217 - page 231 --- --------	1-9