Local vascular pathway for progesterone transfer to the brain after nasal administration in gilts

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SUMMARY

In the present study we examined whether local transfer of intranasally administrated tritiated progesterone (³H-P₄) would increase its concentration in blood supplying the brain and hypophysis in comparison with other organs. Additionally, the effect of estrous cycle on the P₄ transfer was evaluated on isolated gilts’ heads. In the first experiment ³H-P₄ was instilled into the nasal cavities of anaesthetized, immature pigs (n=10). Simultaneous blood samples were collected for radioactivity measurement every minute from the same occluded carotid artery through two catheters; one catheter was pointed towards the head, the other one towards the heart. In eight animals the ratio calculated between the ‘head’ and ‘heart’ samples was significantly (p<0.05) higher than 1 and reached a mean (± SEM) level of 3.23 ± 0.81.
In two animals a much higher ratio was observed. A head/heart ratio > 1 indicates an existence of local transfer of $^3$H-P$_4$ from venous blood to the carotid blood. In the second experiment, heads of 26 mature, cycling gilts were perfused through the right carotid artery with autologous blood. The outflow from the left carotid artery was collected as 1 min samples. $^3$H-P$_4$ was infused into the angularis oculi veins. Transfer of $^3$H-P$_4$ from the venous blood into the arterial blood reached the mean ($\pm$ SEM) level of 4.11 ± 1.08 pg/ml on days 2-4, 3.2 ± 0.70 on days 17-21 and 0.94 ± 0.22 pg/ml on days 15-16 of the estrous cycle. No $^3$H-P$_4$ transfer was observed on days 9-11. These findings demonstrate that nasally administered progesterone can reach the brain in locally higher concentration through the vascular pathway. Moreover, the between-vessel transfer of P$_4$ is significantly affected by the stage of the estrous cycle. 

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INTRODUCTION

A number of natural and synthetic hormones are used for control and optimizing reproductive performance in animals [19]. The bioavailability of these hormones is frequently limited [9, 30] and depends on their stability in gastrointestinal tract, absorption characteristic and hepatic first-pass metabolism. Therefore, a search for new formulations of hormones as well as for their new delivery systems appears to be a good approach to enhance biological activity of exogenous hormones [9, 30]. The intranasal application may be one of the alternate routes for drug delivery [2]. A wide variety of hormones have been recently evaluated for intranasal delivery including progesterone (P$_4$), testosterone, estradiol, posterior pituitary oligopeptides and their analogs, hypothalamic releasing hormones and their derivatives, enkephalins, calcitonin, insulin and interferons [14, 30]. Evidence is emerging that the nasal application of hormones/drugs, in addition to providing an effective way of their administration may also induce a selective distribution of these substances to the brain and hypophysis [6, 7, 24].

It is known that a part of venous effluent from the nasal cavity reaches the angularis oculi vein which, in turn, enters the cavernous sinus [8, 15].
In ungulates (pigs, sheep, cows), the cavernous sinus completely envelops the carotid rete mirabile that is located on the main route of the arterial blood stream to the brain (fig.1; [5, 22]). The cavernous sinus–carotid rete vascular complex forms a huge area characterized by a small diffusion dis-
tance between arterial and venous blood that offers the possibility of heat exchange and transfer of molecules [17]. To date, transfers of LHRH [11, 17, 23], oxytocin [10], β-endorphin [17, 25] testosterone [24], progesterone [17] dopamine [26] and androstenol [16, 27] were demonstrated.

In the present study we intended to ascertain whether local transfer of nasally administrated progesterone will increase its concentration in blood supplying the brain and hypophysis in comparison with other organs. Progesterone has been selected for the examination because of its documented transfer in the cavernous sinus–carotid rete vascular complex [17] and its relatively frequent nasal administration [2, 4, 14].

**MATERIALS AND METHODS**

**Experiment 1**

Experiment 1 was performed to determine if P₄ transfers in vivo from nasal cavity to the arterial blood supplying the brain and hypophysis in pigs.

**Animals**

The study was approved by the Danish Animal Ethics Committee. An experiment was performed on immature female crossbred pigs (n=10, body weight: 48–59 kg) to eliminate the estrous cycle’s effect on both the hormone absorption from the nasal mucosa and the transfer in the cavernous sinus–carotid rete vascular complex. The animals were premedicated with i.m. injections of Dormicum (Midazolam, 5 mg/ml, 1 ml/10 kg) and Sedaperone Vet. (Azaperon, 40 mg/ml, 1 ml/10 kg). Anesthesia was induced with a continuous infusion of Rapino Vet. (Propotol, 10 mg/ml) through an ear vein until an anaesthetic level permitting tracheal intubation was reached. The animal was connected to a respiratory machine. The animal was then changed to gas anesthesia (O₂ 40% and N₂O 60 %). Isoflurane was added and the concentration varied to maintain an anaesthetic level where respiration was not resisted.
Surgical procedure

Each animal was positioned on the back on heated madras. One carotid artery and both external jugular veins were cannulated in such a way to permit the blood circulation throughout the entire experiment. Schematic diagram of the experimental conditions is presented in fig.2. Silastic catheters (o.d. 3 mm, length 60 cm) filled with heparin-saline (10 IU/ml) were inserted into the left common carotid artery and both jugular veins. Both ends of each cannula were inserted through the same incision of the vessel with one tip pointing towards the head and the other towards the heart. The internal jugular veins were ligated. Two catheters (o.d. 1 mm) were inserted 10 cm into both nasal cavities for $^3$H-P$_4$ infusion. The animal was then positioned on the chest. Two ml of heparin solution (5000 IU/ml) was given intravenously. All three vascular catheters were then cut in the middle and blood samples were collected separately from both jugular veins and from the ‘head’ and the ‘heart’ ends of arterial cannula. The catheter inserted into the right jugular vein was attached to the transfusion system and the left jugular catheter was closed. A mixture of saline and heparinized pig blood (1:3; 37°C) obtained from a slaughterhouse was infused with a roller pump with speed rate of infusion regulated according to continuous estimates matching the outflow. The animal was sacrificed with an overdose of mebunal (Nycomed) after the blood collection.

Infusion of $^3$H-P$_4$ and blood samples collection

Two vials, each containing (1,2,6,7-$^3$H)-progesterone (Nycomed-Amer- sham, TRK413, batch 107, 1 mCi, 86.0 Ci/mmol, 269 mCi/mg) were used in the experiments. The original vial solvent was evaporated by a flow of N$_2$. The isotope was dissolved in 275 μl of 99% ethanol, distributed equally to 10 test tubes and kept at –18°C. The ethanol solution was diluted with 2 ml saline, and divided between two disposable syringes. Tritiated P$_4$ in a total dose of 6.1 x $10^7$ dpm (corresponding with 125 ng of P$_4$) was infused through the nasal catheters for 1 minute beginning 2 min after the start of blood collection. Blood samples from the ‘head’ and ‘heart’ ends of
Fig. 2. Schematic diagram of experimental design in experiment 1. The tip of one catheter pointed towards the head (‘head’ end) and the tip of the other one pointed towards the heart (‘heart’ end). $^3$H-progesterone ($6 \times 10^7$ dpm) was infused (1 ml) into each nasal cavity for 1 minute. Parallel blood samples were obtained from the ‘head’ and ‘heart’ carotid catheters before the start of infusion and for 25 min after the end of infusion. To minimize background radioactivity in the circulating blood, the effluent from the head was collected from both jugular veins. Heparinized and heated homologous blood was transfused into the right jugular vein during the whole experimental period. The $^3$H-P$_4$ content was measured in each arterial sample. To evaluate the transfer between the vessels the ratio between radioactivity found in parallel carotid ‘head’ and ‘heart’ samples were calculated. The head/heart ratio $>$ 1 was considered as an indicator of transfer.
carotid cannula and from the jugular vein were collected simultaneously every one minute during the experimental period of 25 minutes. Blood samples were centrifuged and then radioactivity was measured in plasma (see exp. 2).

**Experiment 2**

Experiment 2 was performed to determine the effect of reproductive status on P₄ transfer in the cavernous sinus–carotid rete vascular complex. In this experiment, isolated heads of pigs from different stages of the estrous cycle were used.

**Animals**

The experiments were carried out in accordance with regulations for the care and use of research animals. Studies were conducted during the early luteal phase (days 2-4; n = 6), the middle luteal phase (days 9-12; n = 6), the late luteal phase (luteolysis period- days 15 and 16; n = 7) and the follicular phase (days 17-21; n = 7). Crossbred cycling gilts (100 kg of body weight) were exposed daily to sexually matured, vasectomized boar. The day of mating was designated as Day 0 of the estrous cycle. The day of the estrous cycle was verified by examination of morphology of the ovaries [10].

**Experimental design**

Experiments were performed according to the method described previously [17]. Briefly, the anaesthetized animals were decapitated, silastic catheters were introduced into the jugular veins and the carotid common arteries. The head was perfused with heparinized autologous blood through the right carotid artery. Tritium labeled P₄ (1,2,6,7, ³H-P₄ Amersham International, Buckinghamshire, England, 219 mCi/mg) in a total dose of 7.35 x 10⁷ dpm (corresponding to 150 ng of P₄) was diluted in 10 ml of saline and infused during 5 min into the two angularis oculi veins. Samples of outflow from the left carotid artery were collected at one-minute intervals.
Radioactivity measurement

Collected blood samples were immediately centrifuged. Each duplicate of plasma samples (0.5 ml) was mixed with 10 ml of toluene scintillation cocktail. Control samples (0.5 ml) in five replications, collected before the $^3$H-P$_4$ infusion were measured for background estimation. Radioactivity in each sample was counted for 5 min using a liquid scintillation counter (LS 5000 Beckman Instruments, USA) with a program for automatic quench compensation.

Progesterone identification

To determine whether the radioactivity found in arterial blood represented unaltered $^3$H-P$_4$ the following control experiment was carried out. A concentration of highly specific antibodies against P$_4$ [3] sufficient to bind 61% of radioactive P$_4$ was added to a buffer solution with a known amount (dpm) of $^3$H-P$_4$. Free and bound P$_4$ forms were separated with dextran-coated charcoal. Approximately 82 % of the radioactivity (mean) was identified as immunoreactive P$_4$.

STATISTICAL ANALYSIS

Experiment 1

The mean dpm value of the control blood samples, collected before $^3$H-P$_4$ administration (n=5) was subtracted, as a background value, from the dpm values measured for each min sample. Then the ratio between the ‘head’ and ‘heart’ samples was calculated for the samples taken each minute and is presented as means for 5 minute periods of blood collection. The local increase of P$_4$ in arterial blood supplying the brain was analyzed by one-way ANOVA after log transformation and followed by a Bonferroni test (GraphPad, San Diego, CA, USA).

Experiment 2

Radioactivity level in each experimental sample was corrected for the background value as described above. The differences between background and radioactivity
found in arterial and venous blood samples were estimated by a Bonferroni test. The statistical differences among the mean radioactivity values (overall mean) for four groups of cyclic gilts were estimated by the Bonferroni test (GraphPad, San Diego, CA, USA) for 1 ml of blood sampled during 10 minutes of the experiment, beginning from time when the radioactivity was found in the arterial blood.

RESULTS

Experiment 1

Radioactivity was found in the venous outlet three minutes after the infusion of $^{3}$H-P$_{4}$ began into the nasal cavity. It was much higher in the
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In eight of the animals, during the 11 and 25 minute period after the beginning of the $^3$H-$P_4$ infusion, the ratio between the ‘head’ and ‘heart’ samples was significantly ($p<0.05$) higher than 1. The head/heart ratio reached a mean ($\pm$ SEM) level of $3.23 \pm 0.81$ (fig. 4). In the remaining two animals the head/heart ratio was extremely high and is not included. The head/heart ratio $>1$ was considered as an indicator of progesterone transfer from venous blood to carotid artery.

![Graph showing the ratio between 'head' and 'heart' plasma radioactivity after nasal administration of tritiated progesterone.](image)

*Fig. 4.* The ratio between ‘head’ and ‘heart’ plasma radioactivity (mean $\pm$ SEM) after nasal administration of tritiated progesterone in 8 immature female pigs. $^3$H-$P_4$ was infused for 1 minute into both nasal cavities. Blood samples were collected simultaneously from the ‘head’ and ‘heart’ ends of the catheters inserted into the common carotid artery (1 min total outflow). In each arterial sample radioactivity was corrected for background and then the ratio was calculated. The head/heart ratio $>1$ was considered as an indicator of transfer. Bars with different superscripts are significantly different ($p<0.05$).
Experiment 2

Infusion of $^{3}\text{H-P}_4$ into the cavernous sinus performed on days 2-4, 15-16 and 17-21 of the cycle resulted in a significant increase above radioactivity background value in the carotid artery. No radioactivity in the arterial blood was found on days 9-12 of the estrous cycle (fig.5). This increase started 2-3 min after the beginning of $^{3}\text{H-P}_4$ infusion and reached a maximal level of $3992.3 \pm 3055.8 \text{ dpm/ml (mean \pm SEM)}$ on days 2-4; $772.9 \pm 488.1 \text{ dpm/ml on days 15-16 and 2438.6 \pm 1533.0 dpm/ml on days 17-21}$. Eighty two percent of the radioactivity found was identified as immunoreactive $\text{P}_4$. During the first ten minutes of the significant increase in the arterial radioactivity, the overall mean (± SEM) level of $^{3}\text{H-P}_4$ transferred from the venous blood of the cavernous sinus into the arterial blood of the carotid rete was $4.11 \pm 1.08 \text{ pg/ml on days 2-4; 0.94 \pm 0.22 pg/ml on days 15-16 and 3.2 \pm 0.70 on days 17-21 of the estrous cycle (fig. 6).}$

DISCUSSION

Nasal administered drugs are absorbed into the blood and are partially transported through the olfactory epithelial region to the cerebro-spinal fluid, the olfactory bulbs or, in some cases, into the parenchyma of the brain [13, 29]. Because of that, much interest has been given to the exploitation of the nasal route for delivery of drugs to the brain through a local transfer via the olfactory tract [13, 29]. However, there is still very limited data concerning the use of nasal administration in the selective delivery of exogenous hormones to the brain and hypophysis.

The results of experiment 1 showed that in pigs ‘ordinary’ nasal treatment with $\text{P}_4$ resulted in a higher $\text{P}_4$ concentration in the blood supplying the brain than in other arterial blood. This confirms the existence of a local vascular pathway for steroid transfer from the nasal mucus to the arterial blood of the carotid rete and hence to the brain and pituitary [16, 24, 27]. This pathway includes $\text{P}_4$ absorption from the nasal mucus and further transfer of this steroid from the venous blood of the cavernous
Fig. 5. Time course of $^3$H-P$_4$ level in the arterial blood collected from the carotid rete in isolated heads from gilts (mean ± SEM). Experiments were performed on days 2-4, 9-12, 15-16 and 17-21 of the estrous cycle after infusion of $^3$H-P$_4$ into the cavernous sinus ($n = 6-7$/per group).
sinus into the arterial blood supplying the brain and hypophysis [24]. It may be assumed that such selective distribution of $P_4$ will ensure its higher concentration in the brain and hypothalamus and diminish side effects in other organs.

In monkeys, marked neuroendocrine effects leading to impairment of ovarian function were observed after nasal delivery of $P_4$. Similar doses of $P_4$ would likely to be ineffective when administered by oral or systemic routes [18]. In this species intranasal administration of $\beta$-endorphin resulted in earlier release of prolactin compared with intravenous injection of the same opioid dose [21]. In humans, non-human primates and laboratory

\textit{Fig 6.} Transfer of $^3$H-$P_4$ from the cavernous sinus to the arterial blood in perfused isolated pig head at the different stages of the estrous cycle. The overall mean ($\pm$ SEM) of progesterone concentration was calculated for one ml of arterial blood plasma taken from the carotid artery during 10 minutes of the experiment (starting from the time when significant radioactivity was found in the arterial blood samples). Bars with different superscripts are significantly different: b = $p<0.05$, c = $p<0.01$, d = $p<0.001$. 

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig6.png}
\caption{Transfer of $^3$H-$P_4$ from the cavernous sinus to the arterial blood in perfused isolated pig head at the different stages of the estrous cycle. The overall mean ($\pm$ SEM) of progesterone concentration was calculated for one ml of arterial blood plasma taken from the carotid artery during 10 minutes of the experiment (starting from the time when significant radioactivity was found in the arterial blood samples). Bars with different superscripts are significantly different: b = $p<0.05$, c = $p<0.01$, d = $p<0.001$.}
\end{figure}
animals (rats, rabbits) the area of potential hormone exchange is small due to the absence of carotid rete mirabile [1, 5, 20]. However, Einer-Jensen and Larsen [6, 7] demonstrated the transfer of tritiated water, tyrosine, propanol and diazepam from nasal cavity to the arterial blood supplying the brain in rats. Just recently, a higher concentration of \( P_4 \) in the ‘head’ than the ‘heart’ plasma was found in female rats after infusion of \(^3\)H-\( P_4 \) into the nasal cavities (Einer-Jensen, unpublished). The transfer of intranasal administered hormones/drugs from venous blood to arterial blood supplying the brain was observed in Einer-Jensen’s experiments performed on rats and in our experiment on pigs.

The present results indicate that the ovarian cycle influences the local transfer of \( P_4 \) from the venous blood of the cavernous sinus to the arterial blood. Our data suggest that the efficiency of progesterone transfer is the highest when endogenous level of \( P_4 \) is low. We still do not know whether the increase in \( P_4 \) level in systemic circulation affects its own transfer in the cavernous sinus–carotid rete complex in gilts. In female rats, systemic progesterone treatment 48 and 24 hours before nasal administration of \( P_4 \) had no effect on the efficiency of the steroid transfer (Einer-Jensen, unpublished). An influence of female reproductive status on the local transfer of hormones between vessels of the cavernous sinus-carotid rete complex was demonstrated for LHRH [11, 17], oxytocin [10], β-endorphin [25] and dopamine [26]. The effect of the ovarian/estrous cycle on the transfer of molecules between vessels was also observed in the reproductive tract of women [12], rats [31] and pigs [28]. The efficiency of retrograde transfer of estradiol and rate of retrograde transfer of \( P_4 \) from the ovarian venous blood to the ovarian artery differed among phases of the porcine estrous cycle [28]. However, no direct relationship between the concentration of the steroids in the venous effluent, and the efficiency and rate of the retrograde transfer to the ovary was found [28].

In conclusion, nasally administered progesterone can reach the brain in locally higher concentration through the vascular pathway. Moreover, the between-vessel transfer of \( P_4 \) is significantly affected by the stage of the estrous cycle.
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