Bioavailability of progesterone enhanced by intranasal spraying[1]

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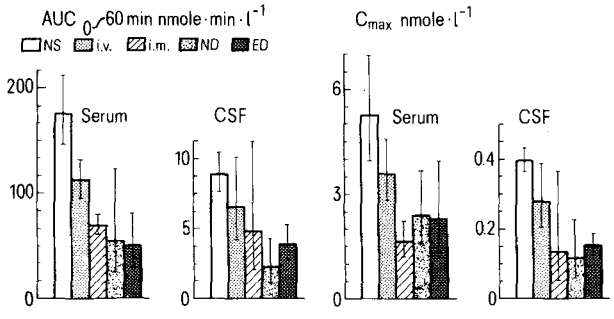
Summary. The bioavailability of progesterone (P) in terms of area under time-concentration curve and maximal concentration in the serum and cerebrospinal fluid was studied in adult ovariectomized rhesus monkeys following the administration of P as a nasal spray, i.v. or i.m. injections, nasal or eye drops. The bioavailability of P in both the body fluids was found to be considerably higher following its being sprayed intranasally.

Exogenous administration of progesterone (P) can impair ovarian and testicular functions. The doses at which such effects are brought about are extremely large and unphysiological if the steroid is administered orally or systemically[2,3]. Recent studies[4,5] reported from our laboratories have shown that comparable effects are also brought about if the steroid is sprayed intranasally but at such low doses as to be ineffective when administered by other, conventional routes. These low-dose effects have been related to the rapid and preferential transfer of the steroid into the cerebrospinal fluid (CSF) following intranasal spraying[6,7] resulting in sufficiently high concentrations of the steroid reaching the brain to interfere with the neuroendocrine mechanisms controlling the secretion of pituitary gonadotropins.

Since the pharmacological effects of a drug are more directly related to its bioavailability than to its administered dose, we extended our studies to compare the bioavailability of P, in terms of 'area under time concentration curve' (AUC)[8] and maximal concentration (C_{max}) following diverse methods of administration. The bioavailability of the steroid was studied in the serum and CSF, the 2 body fluids known to transport P under normal physiological conditions[9].



Area under time-concentration curve (AUC $\int_0^{60\,min}$) and maximal concentration (C_{max}) calculated in the serum and CSF following diverse methods of P administration (dose: 10 µg). Each histogram indicates the geometric mean values and the bars indicate the 95% confidence limits. See text for abbreviations and the table for actual values of P levels at different time intervals.

Methods. 3 healthy adult female monkeys of comparable body weights (5-6 kg) and clinical conditions[10] and exhibiting menstrual cycles of normal duration (25-28 days) were ovariectomized and used 4 weeks later. P (Sigma) was formulated in a solution of ethanol:propylene glycol:water (3:3:4) and administered (dose: 10 µg) as an intranasal spray (NS) using a precalibrated glass atomizer[7], i.v. or i.m. injections, nasal drops (ND) using a tuberculin syringe, or eye drops (ED) using a micropipette. Samples of venous blood and cisternal CSF were collected[7,9] before and at various intervals following the administration of P (table). The 3 monkeys were crossed over between the different routes of administration and the animals were rested for 2 weeks between treatments. The data obtained for AUC and C_{max} following diverse methods of P administration were statistically evaluated by analysis of variance followed be a multiple range test[11].

Results. P levels in serum and CSF following diverse methods of administration are shown in the table.

The AUC $\int_0^{60\,min}$ for serum was maximal following P being sprayed intranasally and it was significantly higher ($p<0.01$) as compared with all other methods of administration. The AUC $\int_0^{60\,min}$ for serum showed a descending order of magnitude in accordance with the sequence of the following routes; i.v., i.m., ND and ED (fig.). The AUC did not differ significantly between these routes.

The AUC $\int_0^{60\,min}$ for CSF (fig.) was considerably higher following NS as compared with all other routes of P administration but this difference was not significant. In the CSF, however, the AUC $\int_0^{60\,min}$ was slightly higher following its instillation as ED as compared with its being

Geometric mean levels (nmoles·l^{-1}) and 95% confidence limits of P in serum and CSF in ovariectomized rhesus monkeys (n=3) following diverse methods of administering the steroid. The steroid was estimated by specific radioimmunoassay as described[13] and using reagents supplied by the WHO under their Quality Control Programme[14]. The characteristics of the assay system have been described[9,13]

Body fluid	Time (min)	NS	i.v.	i.m.	ND	ED
Serum	3	4.46 (2.85-6.97)	3.57 (2.82-4.52)	0.91 (0.24-3.36)	1.07 (0.30-3.83)	2.27 (1.33-3.90)
	9	4.56 (3.46-5.86)	2.85 (2.40-3.3)	0.83 (0.26-2.69)	1.47 (0.69-3.14)	1.67 (1.22-2.28)
	15	3.95 (2.81-5.57)	2.36 (1.97-2.82)	1.11 (0.76-1.62)	1.45 (0.51-4.13)	0.83 (0.53-1.30)
	30	2.52 (2.03-3.11)	1.70 (1.65-1.75)	1.26 (1.13-1.42)	0.59 (0.20-1.76)	0.57 (0.29-1.14)
	60	1.77 (1.44-2.17)	0.88 (0.49-1.58)	1.09 (0.91-1.31)	0.58 (0.27-1.21)	0.37 (0.14-1.00)
CSF	3	0.11 (0.03-0.37)	0.28 (0.20-0.38)	0.01 (0.001-0.08)	0.04 (0.003-0.45)	0.15 (0.12-0.19)
	9	0.29 (0.16-0.55)	0.20 (0.17-0.23)	0.01 (0.001-0.08)	0.10 (0.05-0.20)	0.13 (0.11-0.15)
	15	0.27 (0.17-0.42)	0.16 (0.15-0.17)	0.02 (0.002-0.17)	0.07 (0.05-0.12)	0.06 (0.03-0.13)
	30	0.08 (0.03-0.23)	0.04 (0.01-0.19)	0.11 (0.04-0.31)	0.02 (0.003-0.08)	0.05 (0.03-0.08)
	60	0.08 (0.07-0.09)	0.05 (0.01-0.19)	0.08 (0.04-0.14)	0.002 (0.001-0.005)	0.04 (0.03-0.06)

The background values (geometric mean with 95% confidence limit of 15 samples; 5 samples collected per animal) of P in the samples taken prior to the administration of P were 0.88 (0.77-1.01) nmole·l^{-1} for serum and 0.11 (0.10-0.12) nmole·l^{-1} for CSF; these background values obtained for each of the animals were deducted from the post-treatment values.

administered as ND (fig.). The AUC $\int_0^{60\ min}$ in the serum between ED and ND were almost of a similar order of magnitude (fig.).

C_{max} of P in the serum was maximal following NS (fig.). C_{max} of P was significantly higher ($p < 0.05$) as compared with i.v., ND and ED and also when compared with i.m. ($p < 0.01$).

C_{max} of P in the CSF (fig.) was apparently higher following NS but this level was not significantly different from the C_{max} achieved by i.v. However, C_{max} following NS was significantly higher ($p < 0.05$) as compared with i.m., ND and ED.

C_{max} of P both in the serum and CSF was not statistically different when compared between i.v., i.m., ND and ED.

The best correlation coefficient (r) between serum and CSF levels of P was observed by administering P as ED ($r = 0.827$) followed by NS ($r = 0.676$), ND ($r = 0.671$) and i.v. ($r = 0.613$). Administration of P by i.m. showed the poorest correlation coefficient ($r = 0.202$).

Discussion. The present studies clearly indicate that the bioavailability of P is considerably higher in both the serum and CSF following its being sprayed intranasally and that the enhanced bioavailability is dependent on the method by which P is administered into the nostrils. Previous studies[12] have shown that the bulk of the material sprayed into the nostrils with a glass atomizer is deposited on the olfactory mucosa from where it could enter the subepithelial blood capillaries as well as pass into the CSF.

Transport of the steroid into the blood and CSF across the olfactory mucosa would be a slow process resulting in the steroid also being cleared from the circulation at a slower rate. A slow rate of entry of the steroid into the body fluids accompanied by a slow rate of its clearance could enhance its bioavailability, as indicated in the present studies.

Thus, the present studies not only indicate a novel method of drug delivery which enhances the bioavailability of P but also offer an explanation for the effects observed in terms of impaired ovarian and testicular functions with the intranasal administration of low doses of P[4,5,7].

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PRO EXPERIMENTIS

A simple method enabling standardized handling of small biological objects for light and electron microscopic preparation

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Summary. A glass filtration-apparatus provided with an exchangeable nylon-tissue filter permits collection, fixation, dehydration, staining and preembedding of small biological objects in a simple and rapid way.

Problems arising during the handling of small biological objects for microscopy such as eggs, embryos and small isolated organs of different origins are frequently solved with methods which cannot easily be reproduced[2] for several reasons, such as loss of material due to difficulties in finding the objects after preparation[3], or a high risk of drying[4] or the sticking of the objects during their transport in highly volatile solvents. With the method described here, collection, fixation, dehydration, staining and embedding procedures for light and electron microscopy can be carried out with only minor modifications, whereby optimal conditions for reproducibility are also guaranteed.

Methods and discussion. All procedures take place in a standard vacuum glass filtration apparatus (as used in microbiological methods, Schleicher & Schüll AG, CH-8714 Feldbach, Switzerland) connected by suitable length of tubing to a vacuum membrane pump (Balzers Union, FL-9496 Balzers, Liechtenstein). For biological objects larger than 30 µm, filters consisting of a nylon-tissue disk with a diameter fitting the glass filtration-apparatus and with opening sizes starting from 10 µm (Swiss Silk Bolting Cloth Mfg. Co. Ltd, CH-8027 Zürich, Switzerland) can be used for nearly all of the fixation, staining and dehydration treatments.

After collecting the objects in an appropriate medium, usually a Ringer's solution, the suspension (or isolated larger objects) is given into the cylinder of the glass filtration-apparatus (figure 1). With an appropriate vacuum, the Ringer's solution is sucked away and immediately replaced with the fixative. The objects collected on the filter disk are fixed for an adequate period. The operation is repeated for each subsequent solution according to the chosen treatment. Care must be taken not to overload the apparatus: obstruction of the filter complicates the control of the suction forces, thereby increasing the risk of drying or damaging the objects.