(46) Bhattacharya, S. K., Parmar, S. S. (1985) J. Pharm. Pharmacol. (In Press).
(47) Bhattacharya, S. K., Parmar, S. S. (1985) Pharm. Res. (In Press).
(48) Snyder, S. H., Axelrod, J., Zweig, M. (1965) Biochem. Pharmacol. 14, 831–835.
(49) Neff, N. H., Lin, R. C., Ngai, S. H., Costa, E. (1969) in Advances in Biochemical Psychopharmacology, Vol. 1, Eds. Costa, E., Greengard, P., pp. 91–109, Raven Press, New York.
(50) Sanyal, A. K., Srivastava, D. N., Bhattacharya, S. K. (1981) Indian J. Med. Res. 73, 787–792.
(51) Debnath, P. K., Bhattacharya, S. K., Sanyal, A. K., Poddar, M. K., Ghosh, J. J. (1978) Biochem. Pharmacol. 27, 130–132.
(52) Bhattacharya, S. K. (1982) Res. Comm. Chem. Pathol. Pharmacol. 38, 149–152.
(53) Bhattacharya, S. K., Goodall, W. M., Brumleve, S. J., Parmar, S. S. (1985) Proc. Western Pharmacol. Soc. 28, 217–220.
(54) Henker, D. P., Aiken, J. W. (1980) Eur. J. Pharmacol. 67, 155–158.
(55) Bolla, J., Carson, G. D., Challis, J. R. G. (1977) Prostaglandins 14, 873–879.

RESEARCH ARTICLES

Effect of Dextran on the Release of Gonadotropin-Releasing Hormone (GnRH) Injected into Rats: Plasma GnRH and Gonadotropin Response

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Abstract: Prolonged release of the peptide gonadotropin-releasing hormone (GnRH) from its aqueous solution was achieved by addition of the polymer dextran ($\overline{M}_w \sim 500{,}000$). This effect observed in an *in vitro* system was caused by a decrease of the diffusion coefficient of the peptide. When GnRH was intramuscularly injected into male rats, the addition of dextran to the injected peptide solution led to a prolongation of the GnRH plasma level at the expense of its peak value. This change can be explained by a decrease of the absorption rate of GnRH into blood, which parallels the *in vitro* observation. As a result, the gonadotropin response to GnRH was stronly increased.

Since the identification, characterization, and synthesis of gonadotropin-releasing hormone (GnRH) (1), the hypothalamic decapeptide controlling pituitary gonadotropin secretion, an increasing number of potential uses of this hormone and its superactive agonist analogs has been revealed [reviewed in (2, 3)]. The discovery that GnRH and its agonist analogs not only stimulate but also can inhibit the pituitary-gonadal system by chronic treatment at higher doses [reviewed in (4)] has led to an increasing interest in this peptide. GnRH has recently been proposed as a contraceptive and antitumor agent because of its inhibiting effect on gonadal steroidogenesis (2, 3).

As with other peptides, the therapeutic potential of GnRH is limited by its low metabolic stability, resulting in a very short biological half-life (5, 6), which also applies to the more enzyme-resistant but still degradable analogs (6–8). When GnRH is administered intramuscularly to rats, its plasma level reaches a peak value within a few minutes and then rapidly declines. To prolong an effective plasma level of the peptide, the absorption rate of the peptide from the site of administration into the blood vessel should be lowered.

The aim of this study was to test if a polymer in a dosage form of a peptide, such as GnRH, can retard the release of the peptide. For this purpose we studied (i) the influence of the polymer Dextran T 500 on the diffusion of GnRH out of its solution *in vitro* and (ii) compared this with the *in vivo* effect of the polymer by intramuscularly injecting GnRH solutions with and without the polymer into male rats and measuring the plasma GnRH. Because the polymer led to a prolongation of the GnRH plasma level in these experiments, the plasma levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH), representing the primary biological response to GnRH, were also measured.

Materials and Methods

Chemicals

GnRH was a product of VEB Berlin-Chemie, GDR. [His-^{3}H]GnRH with 0.26–0.56 TBq/mmol (7–15 Ci/mmol) was obtained from our institute (9). Dextran T 500 ($\overline{M}_w \sim 500{,}000$) was from Pharmacia, Sweden.

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In vitro Release of GnRH from Dextran Solutions

The release was determined at 23 °C by a flow-through diffusion method according to Colowick and Womack (10), the details of which are given elsewhere (11). Briefly, the diffusion apparatus consisted of two chambers separated by a cellulose dialysis membrane (Nephrophan®, VEB Filmfabrik Wolfen, GDR) of 2 cm^2, impermeable for the polymer. The upper chamber contained 2 ml of 5×10^{-8} M [His-^{3}H] GnRH and 2×10^{-5} M unlabeled GnRH with and without Dextran T 500, respectively, in a medium consisting of 0.85 % saline, 10 mM sodium phosphate (pH 7.4), 0.01 % HSA, and 0.02 %NaN_3 to prevent microbial growth. GnRH was allowed to diffuse through the membrane into the other chamber of a volume of 0.8 ml through which medium was pumped at a constant flow rate of 0.2 ml per minute. The effluent was collected into fractions of 0.4 ml in which the radioactive concentration of the labeled peptide was determined by liquid scintillation counting. Dextran T 500 was used at a concentration of 10.7 %, the viscosity of the solution being 27 mPa × s at shear rate $D_r \rightarrow 0$ (Couette-type rotation viscosimeter).

To compare the effect of dextran with that of other polymers on release prolongation we defined the parameter

$$Q = [C_2\,(t_{max})_{pol}/C_1(O)_{pol}]/[C_2(t_{max})_{buf}/C_1(O)_{buf}]$$

where $C_2\,(t_{max})_{pol}$ and $C_2(t_{max})_{buf}$ are the maximum concentrations of the tracer in the effluent from the GnRH solutions with and without polymer, respectively, and $C_1(O)_{pol}$ and $C_1(O)_{buf}$ are the tracer concentrations in the upper chamber at the beginning of the diffusion experiment with and without polymer, respectively. Q = 1 means no prolongation, and Q is decreased when the release is prolonged.

Administration of GnRH and GnRH/Dextran to Rats

Adult male Wistar rats weighing approximately 220 g were housed with *ad libitum* access to a standard diet and tap water. The animals were intramuscularly injected into the femur with either 10 µg GnRH in 0.5 ml 0.85 % saline containing 0.01 % HSA to prevent peptide adsorption to glass and plastic surfaces or with the same solution supplemented with 10 % (w/w) Dextran T 500. At the indicated times, the rats were killed by decapitation and the trunk blood was collected in chilled tubes containing heparin. Blood samples were centrifuged and plasma stored at −20°C for subsequent determination of LH and FSH. For the measurement of GnRH, aliquots of the plasma samples were prediluted with buffer and stored frozen. On average 6 rats were used for every experimental point on the plasma level curves.

Hormone Measurements

Plasma levels of GnRH were measured by radioimmunoassay (RIA) using an anti-GnRH serum raised in rabbits against a GnRH-BSA conjugate. The antibody showed cross-reactivity toward C-terminal sequences of GnRH (>5 a a) but only weak reactivity toward the free GnRH acid (1 %) and shorter N-terminal sequences. [^{125}I]iodo-LHRH with a specific activity >55 TBq/mmol (>1500 Ci/mmol) was obtained by radioiodination using the chloramine-T method. Charcoal was used to separate the free and antibody-bound tracer. For the determination of GnRH in the plasma samples, pooled plasma of untreated rats was included in the standard incubations at the same concentration as plasma in the sample incubations to compensate for non-specific interferences by plasma. Details of the RIA are given elsewhere (12).

Plasma LH and FSH were determined by RIA using the rat assay kits kindly provided by the National Pituitary Agency, NIAMDD, Baltimore (USA). Values for LH and FSH are expressed in terms of rat standards LH-RP-1 and FSH-RP-1, respectively. The assay was carried out by the double antibody technique according to A. F. Parlow (NIAMDD). The ^{125}I-tracers were obtained by a modified chloramine-T method using β-mercaptoethanol as stopping reagent. The tracers were purified by Sephadex G-75 gel chromatography. They had a specific activity between 2.5 and 3.5 GBq/mg (67–95 mCi/mg) and were used for about 4 weeks.

Results

Figure 1 shows that the release of GnRH from buffer solution was prolonged when the peptide solution contained 10.7 % (w/w) of the polymer Dextran T 500. This prolonged release was found to be diffusion-controlled because (i) no binding of GnRH to the polymer was observed by equilibrium dialysis and (ii) the release patterns of GnRH with and without dextran were found to be the same when the solution of the upper chamber of the diffusion apparatus was stirred.

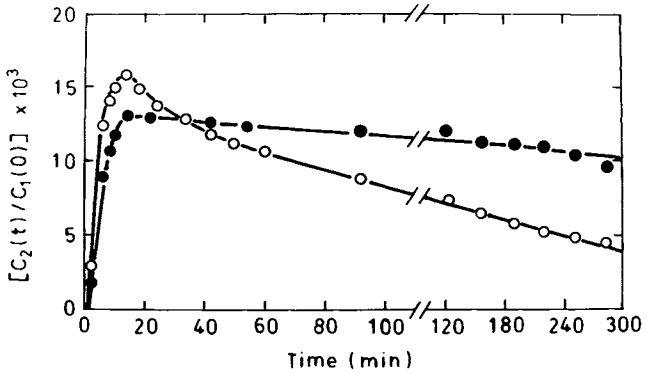

Fig. 1 Effect of 10.7 % Dextran T 500 on the release of ^{3}H-labeled GnRH out of 5×10^{-8} M ^{3}H-labeled GnRH in presence of 2×10^{-5} M unlabeled GnRH in a flow-through cell. The radioactive concentration of the tracer in the effluent at the indicated times $[c_2(t)]$ was related to the concentration of the tracer solution at the beginning of the diffusion $[c_1(0)]$. ○ without dextran, ● with dextran.

Additionally, by using an open-ended capillary technique (13) we found that the self-diffusion coefficient of GnRH ($D = 3.04 \times 10^{-6}$ cm^2/s, in buffer) was lowered by 45 % to $D = 1.66 \times 10^{-6}$ cm^2/s in the presence of 10 % (w/w) Dextran T 500 (14).

Using the parameter Q as defined in "Materials and Methods", we compared the influence of some other polymers, which did not bind GnRH according to equilibrium dialysis measurements (11), on the GnRH release pattern. At equal viscosities of the solutions (η = 27 mPa × s) the following order of increasing prolongation was obtained: Buffer solution (Q = 1), methylcellulose MH 4000 (Q = 1), carboxymethylcellulose 12M3P (Q = 0.93), Dextran T 500 (Q = 0.84). Furthermore, 1 % polyacrylic acid gel of the apparent viscosity ηapp = 12 000 mPa × s at a shear rate of $D_r = 0.47\ s^{-1}$ did not show any effect on the release of GnRH (Q = 1).

From these data Dextran T 500 proved most effective and was, therefore, chosen to study the effect of decreased GnRH diffusion on its pharmacokinetics and biological action in rats.

After a single intramuscular injection of 10 μg GnRH into male rats the plasma GnRH level increased to a maximum within 10 min and, afterwards, declined rapidly, reaching 1 % of its peak value as early as 100 min after injection (Fig. 2).

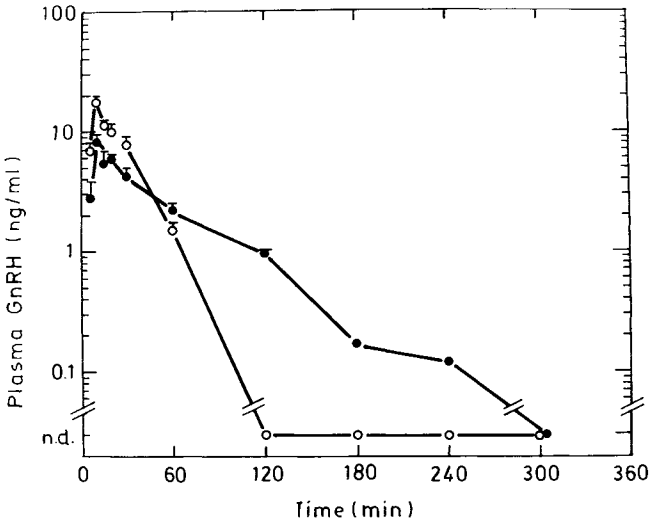

Fig. 2 Plasma levels of GnRH after intramuscular administration of 10 μg GnRH (○) and 10 μg GnRH in 10 % Dextran T 500 (●) into male rats. Each point represents the mean ± SEM. (n.d., not detectable: < 0.1 ng/ml).

The presence of 10 % dextran in the injection solution led to a decrease of the peak height to 45 % of that obtained without dextran. However, the plasma level of GnRH decreased more slowly and beyond 60 min the area under the curve (AUC) was higher by 3-fold (Table I).

The corresponding response of plasma FSH and LH is shown in Figure 3 and 4. Within the first hour, both hormones rose with a slope independent of the presence of dextran in the injection solution. However, in the experiments with dextran, the peak values of FSH and LH were increased by 57 % and 80 %, respectively, and the plasma levels of both hormones declined more slowly. Thus, 6 hours after injection of GnRH/dextran the FSH level is as high as its peak level at 2 hours after injection of GnRH alone and LH is 5 times higher in the experiments with GnRH/dextran at this time. Corresponding to the higher plasma levels using GnRH/dextran the areas under the curve (AUC) of LH and FSH were more than doubled within 6 hours (Table I).

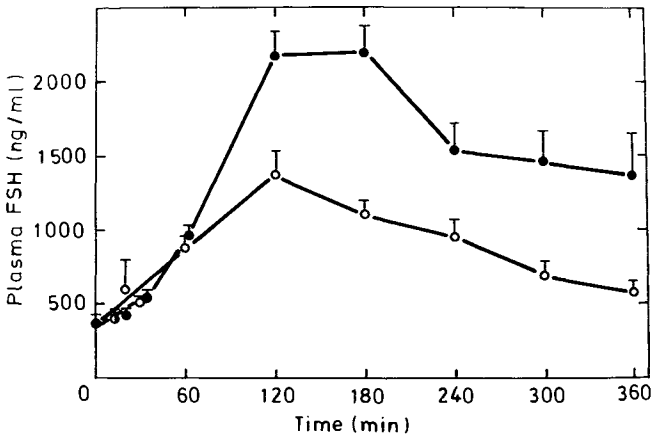

Fig. 3 Plasma levels of FSH (in terms of NIAMDD-rat FSH-RP-1) after intramuscular administration of 10 μg GnRH (○) and 10 μg GnRH in 10 % Dextran T 500 (●) into male rats. Each point represents the mean ± SEM. Between 60 and 360 min the curves were compared by the two-sided paired t-test, and a significant difference was found ($p < 0.01$).

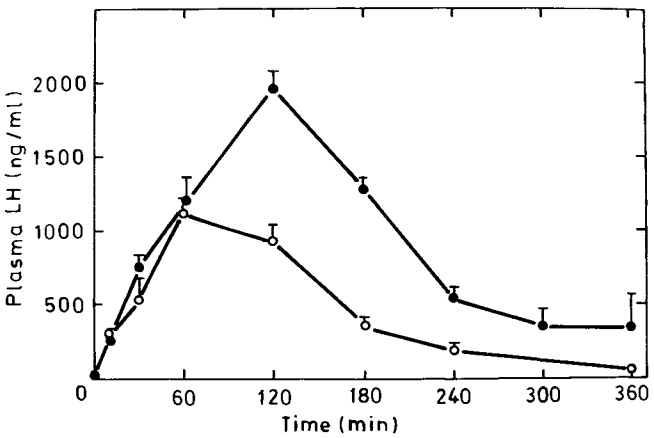

Fig. 4 Plasma levels of LH (in terms of NIAMDD-rat LH-RP-1) after intramuscular administration of 10 μg GnRH (○) and 10 μg GnRH in 10 % Dextran T 500 (●) into male rats. Each point represents the mean ± SEM. Between 60 and 360 min the curves were compared by the two-sided paired t-test and a significant difference was found ($p < 0.05$).

Table I. Relative AUCs (area under the curve) of plasma GnRH, FSH, and LH in male rats during 6 hours after intramuscular injection of 10 μg GnRH in saline and in saline/10 % Dextran T 500, respectively.

	AUC (%)					
	GnRH		FSH		LH	
administration time (min)	GnRH	GnRH + dextran	GnRH	GnRH + dextran	GnRH	GnRH + dextran
0–360 (total)	100	80.6	100	225	100	210
0– 60	91.3	52.6	6.5	7.0	21.1	26.4
60–360	8.7	28.0	93.5	218	78.9	183.6

Discussion

Although soluble polymers have been thought to retard the liberation of drugs from their formulations by increasing the viscosity, such *in vitro* studies on GnRH and other peptides have not so far been described. We found that the polymer Dextran T 500 retarded the release of GnRH from its solution (Fig. 1) because of a decrease in the diffusion coefficient of the peptide. Remarkably, dextran was much more effective than other polymers used in solutions at equal viscosities. Therefore, the practical release prolongation of the peptide from the dosage form by the polymer seems to be independent of the macroscopic viscosity of the solution. To explain this behavior, other factors than the viscosity have to be taken into considera-

tion. Since binding of GnRH to the polymers was excluded, the obstruction effect (15) and the microviscosity (16) in the polymer solutions seem to be of importance.

To study the effect of the polymer Dextran T 500 on the release of intramuscularly injected GnRH into blood and on the gonadotropin response, male rats were used, because it is known that the responsiveness of female rats is dependent on the stage of the estrous cycle (17, 18). The relatively high dose of 10 μg of GnRH was chosen to reach GnRH plasma levels that were near saturation of the gonadotropin response with respect to its peak level. By this procedure, slightly differing plasma GnRH peak values, resulting from dosage forms with and without dextran, respectively, should not have a great influence on the onset of the gonadotropin response, allowing to selectively study the effect of prolonged GnRH plasma levels.

Following the intramuscular injection of GnRH , its plasma level rapidly peaked and then declined (Fig. 2). The change of the time course of the GnRH plasma level by adding Dextran T 500 to the injected solution is typical of a decrease of the absorption rate of the drug from its site of administration into blood, that is, lowering of the peak value and prolongation of an increased plasma level (Fig. 2). The decreased absorption rate can be explained by the decreased diffusion coefficient of GnRH in solutions of dextran as found *in vitro*.

For the dose of GnRH used here and within the first hour, the initial rise of plasma LH and FSH was independent of the plasma level pattern despite of the different peak values and despite the fact that without dextran the amount of plasma GnRH was higher by 1.8-fold. However, after one hour the higher plasma level of GnRH obtained from the dosage form using dextran was obviously the reason for the dramatic increase of the peaks and the amounts of LH and FSH released by the pituitary (Fig. 3, 4; Table I).

As it has been found with human LH, the radioimmunoassay, being a structurally directed assay, does not necessarily measure the biological activity (19). Nevertheless, it seems very likely that the increase in the immunoreactive gonadotropin response as found here principally reflects an increase also in the biological gonadotropin activity because drastic changes in the ratio of biological to immunological activities of human LH have only been found in response to chronic, but not to acute, administration of GnRH analogs (20).

We explain the behavior of the gonadotropin response to the two different plasma level patterns of GnRH with the well-known "self-priming effect" of GnRH. When two pulses of GnRH were administered into female rats the gonadotropin release was found to be larger after the second pulse than that seen after the first injection (21). This effect, which was also found in pubertal and young adult male rats (22), reflects the changes of the pituitary responsiveness during the presence of GnRH. A first injection of GnRH releases a certain amount of LH and FSH and, additionally, increases the responsiveness of the pituitary to a second challenge with GnRH, the maximal responsiveness being about one hour after the priming administration [for a review see (23)].

In our case this would mean that the acute response of LH and FSH to the two kinds of administration is saturated since the high but different GnRH plasma levels led to the same gonadotropin release within the first hour. After that time, at a much lower level of plasma GnRH, the pituitary, now being at a state of higher responsiveness, is assumed to release more LH and FSH in response to the higher GnRH plasma level obtained from the GnRH/dextran dosage form as compared with the level obtained from GnRH administered without dextran.

In summary, when GnRH was intramuscularly injected into male rats, the use of the polymer Dextran T 500 as an additive changed the GnRH plasma level curve in a direction that can be explained by a decrease of the absorption rate of GnRH into blood. This is in keeping with the finding that the diffusion coefficient of GnRH in dextran solutions was found to be lowered. As a result of the measurement of the gonadotropin response to GnRH, the peptide proved to be a hormone where a moderate prolongation of its increased plasma level can lead to a great enhancement of its biological action.

Superactive analogs of GnRH have been found to be eliminated from the circulation by only a slightly lower degree (6, 7). Nevertheless, on the basis of the present findings it may be concluded that their moderately longer presence in plasma could be partly responsible for their higher gonadotropin releasing activity, in addition to their higher receptor affinity (24, 25). Further experiments should show whether the use of polymers in dosage forms of such analogs might be useful to further increase their biological activity, especially when chronic treatment with them is aimed at their inhibitory effects (3).

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References

(1) Matsuo, H., Baba, Y., Nair, R. M. G., Arimura, A., Schally, A. V. (1971) Biochem. Biophys. Res. Commun. 43, 1334–1339.
(2) Fraser, H. M. (1984) Drugs 27, 187–193.
(3) Bex, F. J., Corbin, A. (1984) in Frontiers in Neuroendocrinology, Vol. 8 (Martini, L., Ganong, W. F., eds.), pp. 85–151, Raven Press, New York.
(4) Vickery, B. H. (1982) in Hormone Antagonists (Agarwal, M. K., ed.), pp. 623–657, Walter de Gruyter and Co., Berlin, New York.
(5) Chikamori, K., Suehiro, F., Ogawa, T., Sato, K., Mori, H., Oshima, I., Saito, S. (1981) Acta Endocrinol. 96, 1–6.
(6) Barron, J. L., Millar, R. P., Di Searle (1982) J. Clin. Endocrinol. Metabol. 54, 1169–1173.
(7) Yamazaki, I., Okada, H. (1980) Endocrinol. Japon. 27, 593–605.
(8) Bienert, M., Albrecht, E., Berger, H., Klauschenz, E., Pleiss, U., Niedrich, H., Mehlis B. (1983) Biochim. Biophys. Acta 761, 183–190.
(9) Klauschenz, E., Bienert, M., Egler, H., Pleiss, U., Niedrich, H., Nikolics, K. (1981) Peptides 2, 445–452.
(10) Colowick, S. P., Womack, F. C. (1969) J. Biol. Chem. 244, 774–777.
(11) Lorenz, D., Fechner, K., Mehlis, B. (1985) Pharmazie, in press.
(12) Berger, H., Schäfer, H., Klauschenz, E., Albrecht, E., Mehlis, B. (1982) Anal. Biochem. 127, 418–425.
(13) Wang, J. H. (1951) J. Amer. Chem. Soc. 73, 510–513.
(14) Lorenz, D., Fechner, K., Heinrich, N., Berger, H., Mehlis, B. (1985) Makromol. Chem., Suppl. 9, 163–168.
(15) Mackie, J. S., Meares, P. (1955) Proc. Royal. Soc. (London), Ser. A, 232, 498–509.
(16) Brown, W., Kloow, G., Chitumbo, K., Amu, T. (1976) J. Chem. Soc. Faraday Trans. I, 72, 485–494.
(17) Aiyer, M. S., Fink, G., Greig, F. (1974) J. Endocrinol. 60, 47–64.
(18) Yamazaki, I., Nakagawa, H., Yoshida, K. (1978) Jap. J. Fert. Steril. 23, 405–413.

(19) Storring, P. L., Zaidi, A. A., Mistry, Y. G., Lindberg, M., Stenning, B. E., Dicfalusy, E. (1982) Acta Endocrinol. 101, 339–347.
(20) Evans, R. M., Doelle, G. C., Lindner, I., Bradley, V., Rabin, D. (1984) J. Clin. Invest. 73, 262–266.
(21) Aiyer, M. S., Chiappa, S. A., Fink, G. (1974) J. Endocrinol. 62, 573–588.
(22) Nazian, S. J., Mahesh, V. B. (1979) Neuroendocrinology 29, 313–322.
(23) van Rees, G. P., Schuiling, G. A., de Koning, J. (1983) in Neuroendocrine Perspectives, Vol. 2 (Müller, E. E., Mac Leod, R. M., eds.), pp. 173–198, Elsevier Science Publishers B. V.
(24) Clayton, R. N., Shakespear, R. A., Duncan, J. A., Marshall, J. C. (1979) Endocrinology 105, 1369–1376.
(25) Reeves, J. J., Seguin, C., Lefebvre, F.-A., Kelley, P. A., Labrie, F. (1980) Proc. Natl. Acad. Sci U.S.A. 77, 5567–5571.

Cutaneous Absorption of Indomethacin From Two Topical Preparations in Volunteers

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Abstract: The percutaneous absorption of indomethacin in 0.5 % or 1 % solution or 1 % gel at a dose of 50 mg or 100 mg indomethacin was compared in a randomized complete block design in seven healthy volunteers. The formulations were applied over an area of 12 dm^2 under an 8 h occlusion dressing. In addition, in the same volunteers the plasma concentration curves were determined after a single oral dose of 50 mg indomethacin. Indomethacin and some of its metabolites were determined with modified, existing assays using HPLC-fluorescence or gas chromatography-mass spectrometry. On the basis of a newly developed method, it was possible to separate and quantify *O*-desmethylindomethacin and *N*-deschlorobenzoyl-*O*-desmethylindomethacin. After cutaneous administration of the two drug formulations, peak indomethacin plasma concentration of 95 ng/ml and 130 ng/ml were found between 4 and 8 h; the cutaneous bioavailability was approximately 20 % of the oral dose, as judged by comparing the areas under the plasma concentration time curves (AUC) and the amount of metabolites excreted into the urine. Percutaneous absorption did not change the metabolic pattern in the urine that is obtained after oral administration.

In recent years there has been a dramatic development of interest world-wide in the dermal and transdermal delivery of drugs. The prime interest has centered on drugs such as scopolamine (1), nitroglycerine (2), and clonidine (3). All of these substances are delivered for systemic effect since the required blood levels to induce activity are extremely small.

Recently two products have appeared on the market containing indomethacin for application to the skin for local treatment of rheumatic and other pain (4). It is the aim of this study to determine if a substance such as indomethacin will penetrate the skin and to what degree the intact and metabolized drug appears in the blood and is excreted into the urine of volunteers. It must be clear that this study is concerned only with the absorption process and metabolism and in no way purports that the absorbed drug substance is present in quantities that will elicit a therapeutic response.

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Materials and Methods

Experimental Design

Volunteers. Seven healthy male volunteers aged between 19 and 39 years, median 21 years, with a mean body weight of 72.7 ± 7.1 kg participated in the trial. Subjects with any type of skin disorder were excluded. According to the requirements the volunteers received a pre- and a post-medical examination, signed an informed consent, and were not allowed to take any drugs during the trial period.

Trial Products. The two solutions manufactured by Luitpold-Werk contained 0.5 % or 1.0 % indomethacin (Elmetacin®, 0.5 %: batch-No. V 80 033; 1 %: batch-No. V 80 023 in an alcoholic solution). The gel was commercially available (Amuno®-Gel, MSD-Frosst-Pharma, batch-No. 28 356), containing 1 % indomethacin. The commercial tablet (Amuno®, MSD Pharma, batch-No. 28 202) contained 50 mg indomethacin.

Trial Protocol. The trial was a cross-over of each of the cutaneously applied products in the seven volunteers with a washout period of at least two weeks between each. The products were applied to subjects 1 to 7 in a random sequence that was determined before initiation of the trial as indicated in Table I.

At the day of the first drug application each volunteer was allocated at random to a number to which a specific color was attached. The color code was unknown to the laboratory technician of the company. When this randomized block design was completed, each subject received a single oral dose of 50 mg indomethacin (140 μmoles) after a wash-out period of two weeks. In order to obtain standard conditions the products were applied to each volunteer for each trial on the same day at 5 min intervals.