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The influence of azone on the transdermal penetration of the dopamine D_2 agonist N-0923 in freely moving rats

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Summary

The transdermal penetration of the dopamine D2 agonist, N-0923, was studied in freely moving male albino Wistar rats, after cutaneous dosing of 2 μmol cm^{-2} in a carbomer gel A silicone patch with an effective area of 0 65 or 1.3 cm^2 was filled with 100 or 200 mg gel, respectively. The patch was attached to the rat skin using histoacrylic glue. The drug was tested as N-0923 HCl The enhancing effect of azone was studied by adding 5% of the penetration enhancer to the gel A second enhancement study was performed after pretreatment of shaved rat skin with a solution containing 5% azone at various time points, prior to administrations of drug The pretreatment solution contained ethanol-propyleneglycol-water-azone (60 20 · 15 · 5 v/v). The dermal penetration of N-0923 HCl from the carbomer gel resulted in plasma levels which were relatively low and often at or just above the limit of quantitation of the assay (10 pmol ml^{-1}) Adding 5% azone to the gel resulted in undetectable plasma levels, indicating the drug not being released from the carrier Pretreatment of the rat skin with a solution containing 5% azone resulted in a 3-fold increase in the flux vs controls Plasma levels were detectable up to 10 h after application. All pretreated animals showed stereotypy, indicating that adequate amounts of the drug reached the brain.

Introduction

The racemic drug, (±)-2-(*N*-propyl-*N*-2-thienylethylamino)-5-hydroxytetralin hydrochloride (N-0437), synthesized by Horn et al. (1985) proved to be a potent and selective dopamine D2 agonist (Van der Weide et al., 1988). The individual enantiomers showed greatly different activities at the dopamine D2 receptor. The highest dopaminergic activity was found to reside in the (−) enantiomer which is known as N-0923. Due to the aromatic hydroxyl group the drug metabolizes very rapidly, mainly to glucuronide and sulfate conjugates (Gerding et al., 1990). In rats, N-0437 is absorbed to an extent of at least 40% after oral dosing but the absolute bioavailability is reduced to maximally 0.5% (Swart and De Zeeuw, 1992). Alternative routes of administration such as the buccal or nasal route are characterized by rapid onset and relatively short duration of pharmacodynamic effects (Swart et al.,

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1992a). The half-life of the drug in rats was found to be in the order of 2–3 h (Swart and De Zeeuw, 1992; Swart et al., 1992b).

Pharmacological studies after dermal application of the racemic mixture, N-0437, to rats with unilateral 6-OHDA lesions in the striatum (Den Daas et al., 1990) or to MPTP-treated marmosets (Löschmann et al., 1989) resulted in a positive response for both models. The 6-OHDA lesioned rats showed contralateral turning and the MPTP-treated marmosets displayed an increase in locomotor activity. Timmerman et al. (1989) reported a 60% decrease in dopamine release after dermal application of 10 μmol kg^{-1} N-0923 to rats using brain dialysis. The maximal decrease lasted for at least 8 h, whereas the total duration following transdermal administration was about 13 h. Therefore, dermal dosing was proposed to enhance the bioavailability by circumventing the oral first-pass effect in order to achieve plasma levels in the therapeutic range. Recently, we described the in vitro penetration of N-0923 through full-thickness rat skin (Swart et al., 1992c), in which we found that in vivo pretreatment of the skin with the penetration enhancer azone considerably increased transdermal penetration in vitro. Application of azone to the dosing vehicle without pretreatment had no effect. Pharmacological and toxicological studies showed that azone possesses a safe toxicological profile (Stoughton, 1982, 1983; Wiechers et al., 1990, 1992).

In this study we have investigated the penetration enhancing effect of azone in freely moving rats after treatment of the skin prior to dosing as well as after dosing with an azone-containing formulation.

Materials and Methods

Chemicals

The drug *S*-(−)-2-(*N*-propyl-*N*-2-thienylethylamino)-5-hydroxytetralin hydrochloride (N-0923 · HCl) and 1-dodecylazacycloheptan-2-one (azone) were obtained from Whitby Research (Richmond, VA, U.S.A.). The enantiomer, N-0923 · HCl, was prepared according to the method described by Ten Hoeve and Wijnberg (1985) and converted to the HCl salt according to the method of Horn et al. (1985). The pK_a value for the tertiary amine group was 7.9. The drug (±)-2-(*N*-propyl-*N*-2-*p*-fluorophenylethylamino)-5-hydroxytetralin hydrochloride, used as the internal standard (IS), was kindly donated by the Department of Medicinal Chemistry, University Centre for Pharmacy, Groningen, The Netherlands. The enantiomeric purity of N-0923 was at least 99.9%, as determined by the method of Witte et al. (1992) and the purity of the IS was evaluated to be at least 98%, by means of HPLC and TLC. 1-Octanesulfonic acid (sodium salt) was supplied by Eastman Kodak (Rochester, NY, U.S.A.) and methanol, HPLC grade, by Baker (Deventer, The Netherlands). Halothane was obtained from ICI Farma (Rotterdam, The Netherlands). Propylene glycol (PG) was purchased from Brocacef (Maarssen, The Netherlands). Histoacryl glue was obtained from B. Braun Melsungen AG (Melsungen, Germany) and Carbopol 934 was from OPG (Groningen, The Netherlands). All other chemicals were analytical reagent grade and obtained from Merck (Darmstadt, Germany). Throughout the study, de-ionized water was used (Milli-Q purification system, Millipore, Bedford, MA, U.S.A.).

Stock solutions

Stock solutions of 2.8 μmol ml^{-1} N-0923 · HCl and IS were prepared in Milli-Q water. Appropriate dilutions of the drug were made daily in drug-free plasma to provide calibration samples with concentrations of 7 pmol ml^{-1}–1.5 nmol ml^{-1}.

Cutaneous carbomer gel

The ethanol-containing carbomer gel was prepared according to the Formularium of Dutch Pharmacists (Cox, 1989). N-0923 · HCl was dissolved in the ethanol fraction and the latter mixed with the gel to give a gel concentration of 13.5 μmol g^{-1}. A second carbomer gel was prepared, containing 13.5 μmol g^{-1} N-0923 and 5% (v/v) azone. The final pH of the gels was approx. 5, and they were stored in the dark at 4 °C.

Chromatography

The chromatographic system consisted of a model SP 8800 HPLC pump (Spectra Physics, San Jose, CA, U.S.A.), and an autosampler model 460 fitted with a 500 μl loop, (Kontron Instruments, Basel, Switzerland). Detection was performed using an amperometric detector, with a working potential of 0.75 V, model AMOR, equipped with a glassy carbon electrode (Spark Holland, Emmen, The Netherlands). Peak heights were recorded with a 3396A reporting integrator (Hewlett Packard, Avondale, PA, U.S.A.).

Separation was performed using a 150 × 3.8 mm i.d. column packed with 4 μm Nova Pak C18 (Waters, Milford, MA, U.S.A.). The isocratic mobile phase used for the separations contained 65% 50 mM sodium phosphate pH 5.5 and 35% acetonitrile. 1-Octanesulfonic acid and sodium chloride were added at final concentrations of 3.5 and 5 mM, respectively. After filtering the eluent through a 0.20 μm type RC-58 membrane filter (Schleicher and Schuell, Dassel, Germany), the mobile phase was degassed in an ultrasonic bath for 15 min. During the measurements the eluent was continuously de-aerated with helium. The flow rate was 1.0 ml min^{-1}. The column and detector were thermostatted at 25 °C with a circulating water bath (MGW Lauda, Germany), to minimize fluctuations in background current.

When not in use, the chromatographic system and the detector were continuously flushed with eluent at a flow rate of 0.2 ml min^{-1}.

Sample preparation for the analysis of free N-0923 base

The samples were prepared on the basis of the method described earlier for the analysis of the racemic N-0437 in human plasma (Swart et al., 1990). Minor modifications were introduced for rat plasma extraction.

To 100 μl plasma, 100 μl water containing the IS were added and the mixture was vortex mixed for 1 min. Subsequently, 800 μl of water were added and vortex mixing was repeated. When spiking a sample, 100 μl of water containing the required amount of N-0923 and/or IS were added to 100 μl of biofluid, followed by the above procedure.

Bond Elut® extraction columns, type Si (silica gel), bed volume 2.8 ml (Varian, Harbor City, CA, U.S.A.), were used in combination with a Vac Elut® vacuum control station. Each column was pretreated by subsequent washings with 2.5 ml methanol, 2.5 ml water, 2.5 ml dichloromethane and 5 ml water. After removing the final washing, 950 μl of the diluted sample were transferred to the washed column and a gentle vacuum was applied (80 kPa). The columns were rinsed with 5 ml water under 80 kPa. During the washings, care was taken that the column did not run dry and that it remained submersed at all times. The columns were placed in a plastic tube and centrifuged for 3 min at 635 × g, to remove any remaining droplets of water. Elution of the adsorbed N-0923 and IS was achieved by passing 7.5 ml dichloromethane through the column under a pressure of 80 kPa. The eluate was collected in a 10 ml Pyrex glass tube (Sovirel®), after which 25 μl acidified methanol were added (40 mM acetic acid in methanol). The sample was vortex mixed and the organic layer was evaporated to dryness within 30 min, using a vacuum concentrator at 1500 rpm and 40 °C (Uni Equip, Martiensried, Germany). The residue was reconstituted in 250 μl eluent by vortex mixing for 1 min to achieve complete dissolution and 200 μl of the sample were injected on the HPLC-column.

Animals

Male Albino Wistar rats, weighing 250–300 g (CDL, Groningen, The Netherlands) were used. The jugular vein was cannulated according to the method of Steffens (1969). After surgery, the animals were housed individually in plastic cages (35 × 35 × 40 cm) and permitted tap water and standard laboratory chow ad libitum. Lights were on from 07.00 to 19.00 h. The recovery period was at least 1 week.

Transdermal administration

Hair of the neck region was removed 2 days before the experiments with an electric razor without damaging the skin. The shaven skin was pretreated as follows: At four points, 49, 42, 25, and 18 h before the cutaneous dosing, 0.1 ml of the pretreatment solution A or B was spread on

the skin (5 cm^2) with a glass rod.

Solution A consisted of 60% ethanol-20% PG-20% water (control); solution B consisted of 60% ethanol-20% PG-15% water-5% azone. Just before dosing, the animals were slightly anaesthetized with ether for 5 min, and a silicone patch with a specific area of 0.65 or 1.3 cm^2 containing 100 or 200 mg drug-carbomer gel, respectively, was attached to the skin with histoacrylic glue. The final dosing for both patches was 2 μmol cm^{-2}. Blood samples, 200 μl each, were collected through the cannula in the jugular vein using plastic disposable syringes over a 10 h period. After heparinization with 2 μl 500 IU ml^{-1} heparin, the samples were centrifuged for 5 min at 10000 rpm in a Biofuge A (Heraeus Christ, Osterode am Herz, Germany). The clear plasma was stored at $-20\,^{\circ}C$ until analysis. Since the total volume of blood taken per experiment was approx. 2.5 ml, 1 ml rat donor blood was given at 300 min to compensate for this loss.

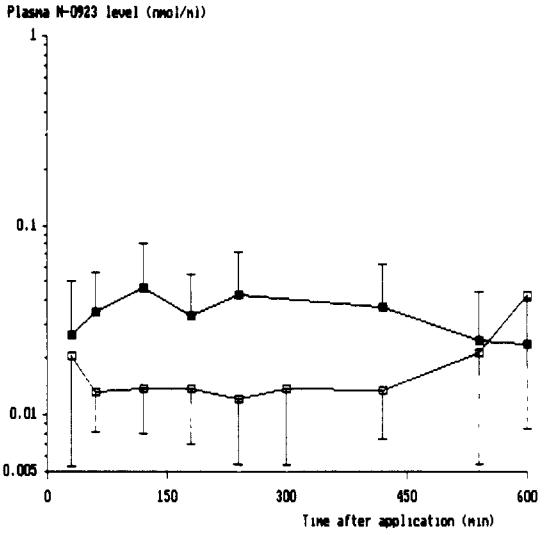

Fig 1 Mean curves of the plasma concentration-time profiles after application of the small patch. Pretreatment of rat skin with (□) 60% ethanol, 20% PG, 20% water, (■) 60% ethanol, 20% PG, 15% water, 5% azone

Results and Discussion

The N-0923 penetration data, flux in nmol ml^{-1} h^{-1}, lag time in h, and the percentage of the dose absorbed in %, are given in Table 1. The flux (F) was calculated from the following equation: $F = Cl_p \cdot C_{ss}/A$, where Cl_p is the total plasma clearance in ml h^{-1}, C_{ss} the plasma concentration at steady state in nmol ml^{-1}, and A the effective area of the patch in cm^2. The percentage of the dose absorbed up to 600 min after dosing was calculated using a deconvolution method (Proost, 1985, 1987). The pharmacokinetics of N-0923 are characterized by an 'inverse' dose dependency, e.g., the elimination half-life decreases with increasing doses. On the basis of the relatively low plasma concentrations found in this study, the calculations based on pharmacokinetic data obtained after an i.v. bolus of 0.5 μmol kg^{-1} to freely moving male rats were considered to provide the most reliable estimation of absorption data (Swart et al., 1992b). Figs 1 and 2 show

TABLE 1

Estimated concentrations at steady state and fluxes of N-0923 (mean values ± S D)

Patch area (cm^2)	Use of azone	C_{ss} (pmol ml^{-1})	Flux (nmol cm^{-2})	Lag time range (min)	Absorption (%)	n
1 3	none	23 ± 17	1 4 ± 0 5	17– 39	0 5 ± 0 2	3
1 3	5% in gel	nd	nd	nd	nd	3
1 3	5% pretreatment	61 ± 43	4 5 ± 2 9	32–166	1.6 ± 1 2	5
0 65	none	13 ± 5	2.0 ± 0.8	nc	0.8 ± 0 3	3
0 65	5% pretreatment	32 ± 15	4 8 ± 2 5	0– 30	3.6 ± 3.2	5

nd, not detectable; nc, not computable

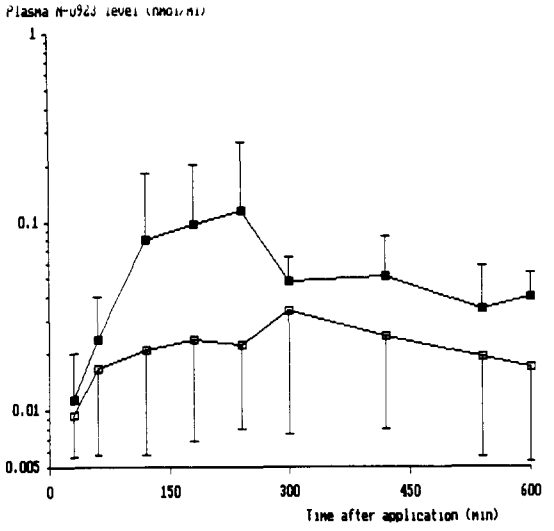

Fig 2 Mean curves of the plasma concentration time profiles after application of the large patch Pretreatment of the rat skin with: (□) 60% ethanol, 20% PG, 20% water; (■) 60% ethanol, 20% PG, 15% water, 5% azone.

the mean plasma concentration-time curves of N-0923 after application of the small and large patches, respectively. Both Figs 1 and 2 demonstrate that the plasma levels of the controls are at or just above the quantitation limit of the assay (10 pmol ml^{-1}). Based on this quantitation limit, the minimal required or detectable flux was calculated to be 1.6 nmol cm^{-2} h^{-1} for the small patch and 0.8 nmol cm^{-2} h^{-1} for the large one. The mean fluxes found for the controls, 2.0 ± 0.8 nmol cm^{-2} h^{-1} for the large patch and 1.4 ± 0.5 nmol cm^{-2} h^{-1} for the small one, are slightly above the minimal values.

Animals pretreated with solution A and to which a 1.3 cm^2 patch was attached containing a gel formulation with 5% azone showed neither detectable plasma levels nor stereotypy. Therefore, it may be concluded that N-0923 is not being released from the carrier. Similar results were obtained in in vitro transdermal penetration experiments (Swart et al., 1992c): introducing azone into the vehicle resulted for N-0923 base in a large decrease in in vitro flux and for N-0923 · HCl in a flux which declined to almost zero after 10 h.

Pretreatment of the shaven rat skin with solution B resulted in an increase of average steady-state plasma concentrations of about 3-fold for the small as well as the large patches. The estimated flux for the large patch increased 3-fold to 4.5 ± 2.9 nmol cm^{-2} h^{-1} (Student's t-test, vs controls $p = 0.066$) and that for the small patch increased about 2.4-fold to 4.8 ± 2.5 nmol cm^{-2} h^{-1} (Student's t-test, vs controls, $p = 0.087$). The increase in flux of N-0923 through azone-pretreated rat skin is in line with the previously observed in vitro data.

The lag times for the large patch ranged from 17 to 39 min for the non-pretreated rats and from 32 to 166 min for the pretreated skin, respectively. The lag time for the small patch ranged from 0 to 30 min for the pretreated skin. Due to the low plasma concentrations after application of the small patch on the non-pretreated skin, no reliable lag times could be fitted. The lag times in vivo appeared to be much shorter than those in vitro, which may be explained by the so-called 'sink' conditions due to the high degree of tissue binding of N-0923 (Swart et al., 1992d) and the blood circulation through the epidermis in vivo.

The percentage of dose absorbed of the controls during the 10 h of penetration was found to be 0.5 ± 0.2 and $0.8 \pm 0.3\%$ for the large and small patch, respectively. The minimal required absorption, based on the quantitation limit, was 0.54% for the large patch and 0.27% for the small one. The in vivo pretreatment of the skin with azone resulted in an increase in the percentage absorbed of about 5-fold to $3.6 \pm 3.2\%$ for the small patch and a 3-fold increase to $1.6 \pm 1.2\%$ for the large one.

A slight stereotypy was observed as soon as the animals of which the skin was pretreated with azone became conscious, indicating that pharmacologically relevant amounts of the drug reached the brain. These findings show that N-0923 can be delivered via the transdermal route and therefore may be an attractive alternative to circumvent first-pass metabolism and to achieve therapeutically relevant systemic plasma concentrations. Pretreatment of rat skin with a solution containing sufficient amounts of azone prior to application may substantially enhance the penetration of the drug.

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