when both enkephalin degrading enzymes, enkephalinase and aminopeptidase were completely inhibited in the presence of the mixture of dipeptides[7,10]. Lindberg and Dahl[11] did not detect a kyotorphin-induced Met-enkephalin release when using bacitracin as a peptidase inhibitor. However, bacitracin (about $4.3 \times 10^{-5}$ M) might be a less potent peptidase inhibitor than the mixture of dipeptides (each 1 mM)[10], therefore the added kyotorphin and released Met-enkephalin may have been degraded during the superfusion.

It has been reported by several investigators that endogenous opioid peptides are involved in the response to the nociceptive stimuli[12,13]. Most recently Kuraishi et al.[14] demonstrated that during the nociceptive stimuli, Met-enkephalin is released from the nucleus reticularis gigantocellularis of the rat medulla oblongata, a most sensitive site to the analgesic effects of morphine and enkephalin[15,16], thereby suggesting that enkephalin may play a role in pain regulation. Our present data suggest that both kyptorphin and D-kyotorphin produce analgesic effects by the induction of Met-enkephalin release. However, D-kyotorphin shows higher in vivo analgesic activity than does kyotorphin[8,9], despite the finding that both dipeptides are equipotent regarding enkephalin release. In preliminary experiments it was shown that kyotorphin ($10^{-5}$-$10^{-3}$M) was completely degraded within 10 min when incubated with $S_2$-fraction or $P_2$-fraction of the rat brain at 37 °C. In contrast, D-kyotorphin ($10^{-5}$M) remained intact after incubation for 60 min in these brain fractions. The more powerful action of D-kyotorphin can therefore be explained by the fact that it is more stable in the brain fractions than kyotorphin.

Thus, D-kyotorphin can probably serve as a useful tool for investigating the involvement of enkephalin in a variety of behavioral situations and may even represent the prototype of a new class of pharmacological agents.

1 Acknowledgment. We thank M. Ohara of Kyushu University for comments on the manuscript.
2 Shiomi, H., Ueda, H., and Takagi, H., Neuropharmacology *20* (1981) 633.
3 Takagi, H., Shiomi, H., Ueda, H., and Amano, H., Nature, Lond. *282* (1979) 410.
4 Ueda, H., Amano, H., Shiomi, H., and Takagi, H., Eur. J. Pharmac. *56* (1979) 265.
5 Rackham, A., Wood, P.L., and Hudgin, R.L., Annual Meeting of Socienty for Neuroscience, USA 1981, abstracts, p. 765.
6 Satoh, M., Kawajiri, S., Yamamoto, M., Akaike, A., Ukai, Y., and Takagi, H., Neurosci. Lett. *16* (1980) 319.
7 Shiomi, H., Kuraishi, Y., Ueda, H., Harada, Y., Amano, H., and Takagi, H., Brain Res. *221* (1981) 161.
8 Takagi, H., Shiomi, H., Ueda, H., and Amano, H., Eur. J. Pharmac. *55* (1979) 109.
9 Laubie, M., and Schmitts, H., Eur. J. Pharmac. *71* (1981) 401.
10 Sullivan, S., Akil, H., Blacker, D., and Barchas, J.D., Peptides *1* (1980) 31.
11 Lindberg, I., and Dahl, J.L., J. Neurochem. *36* (1981) 506.
12 Jacob, J.J., Trembly, E.C., and Colonbel, M.C., Psychopharmacologia *37* (1974) 217.
13 Woolf, C.J., Brain Res. *190* (1980) 578.
14 Kuraishi, Y., Sugimoto, M., and Takagi, H., in: Advances in endogenous and exogenous opioids, p. 182. Eds H. Takagi and E.J. Simon. Kodansha-Elsevier, Tokyo/Amsterdam 1981.
15 Akaike, A., Shibata, T., Satoh, M., and Takagi, H., Neuropharmacology *17* (1978) 775.
16 Takagi, H., Satoh, M., Akaike, A., Shibata, T., and Kuraishi, Y., Eur. J. Pharmac. *45* (1977) 91.

## Bioavailability of norethindrone in women and rabbits after administration of norethindrone acetate through the subcutaneous route

N.N. Sarkar, K.R. Laumas and V. Hingorani[1]

*Department of Reproductive Biology and Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi-110029 (India), 10 July 1981*

*Summary.* The levels of available norethindrone were found to be similar in women and rabbits after s.c. administration of crystalline and amorphous norethindrone acetate respectively, and this study indicates that the rabbit may be used as an animal model for further investigation with this contraceptive steroid.

Norethindrone (NET), a biologically active metabolite of norethindrone acetate (NETA) is reported to be available in the plasma of animals[2] and women[3,4] after s.c. insertion of a silastic implant containing NETA. The present study is a comparison between the availability of NET in the serum of women and rabbits after administration of allomorphs of NETA through the s.c. route, to examine whether the rabbit could be used as an animal model for pharmacological and toxicological studies of this contraceptive steroid.

*Materials and methods.* Polydimethyl siloxane (silastic) implants-D were prepared with 40 mg crystalline or amorphous NETA according to the method described elsewhere[5]. Groups of 6 women and 6 New Zealand breed female albino rabbits were selected for this study. A single silastic implant containing crystalline NETA was s.c. inserted in the thigh of each woman under local anesthesia with 2% xylocaine. Similarly, an implant containing amorphous NETA was inserted in the thigh of a hind leg of each rab-

Serum NET levels (ng/ml) after insertion of an implant containing 40 mg NETA

| | | Treatment period (in weeks) 1 | 2 | 3 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Women mean | (n=6) | - | 1.2 | - | 1.7 | 1.4 | 1.5 | 1.2 | 1.2 | 1.0 | 0.9 | - | |
| Women mean ± SD | | - | 0.5 | - | 0.6 | 0.4 | 0.8 | 0.5 | 0.4 | 0.2 | 0.6 | - | |
| Rabbits mean | (n=6) | 3.1 | 3.8 | 1.4 | 1.4 | 1.1 | 0.9 | 1.0 | 1.1 | 0.8 | 1.0 | 0.7 | $p > 0.1$* |
| Rabbits mean ± SD | | 0.8 | 2.3 | 0.3 | 0.6 | 0.4 | 0.2 | 0.5 | 0.1 | 0.1 | 0.2 | 0.4 | |

n, Number of samples, *not significant.

bit. Blood samples were collected fortnightly up to the 16th week in women and 18th week in rabbits. Serum samples were analyzed for the peripheral NET level by a radioimmunoassay technique[2]. The statistical evaluation (analysis of variance) of results was performed using a programmed memory in a Hewlett-Packard calculator (model Hp-09810).

*Results.* In women, the mean serum NET level (table) fluctuated between 1.4 and 1.5 ng/ml up to the 8th week. Thereafter, it was almost constant around 1.2 ng/ml up to the end of the treatment period. In rabbits, the NET level followed a similar pattern except in the 1st and 2nd weeks, where it was quite high, yet no statistical significant difference was observed between the mean serum NET levels in women and rabbits in this study.

*Discussion.* The present study revealed that NET was equally bioavailable in both women and rabbits after insertion of implants containing allomorphs of NETA. A similar value for NET was also observed in rabbits with implants containing crystalline NETA[2] except that an initial surge was found in the present study. This discrepancy probably resulted from the difference in the pre-equilibrium diffusion rate between crystalline and amorphous NETA through the wall of the silastic implant. A serum NET level of about 1 ng/ml has also been reported in women with an implant of NETA[3], and this level appeared to have a therapeutic effect for the control of conception in women[6]. Moreover, the NET levels attained in rabbits were found to be almost identical with those observed in women. These findings, therefore, suggest that the rabbit may serve as an animal model for further study with this contraceptive steroid.

1 Reprint requests to V.H., Dept. of Obstetrics and Gynecology A.I.I.M.S., New Delhi-110029 (India).
2 N.N. Sarkar, V. Laumas and K.R. Laumas, Endocr. Jap. *27*, 653 (1980).
3 S.G. Hillier, P. Jha, K. Griffiths and K.R. Laumas, Contraception *15*, 473 (1977).
4 V. Goyal, U.M. Joshi, V.K. Naik and P.K. Devi, Contraception *17*, 375 (1978).
5 S. Bhatnagar, U.K. Srivastave, D. Takkar, V.L. Chandra, V. Hingorani and K.R. Laumas, Contraception *11*, 505 (1975).
6 V. Laumas, A.K. Jain, P. Jha, S.A. Rahman, D. Kumar, B.K. Malik. N.N. Sarkar, D. Takkar, V. Hingorani and K.R. Laumas, Contraception *23*, 211 (1981).

## Antibacterial properties of several drug categories

A. Delitheos, S. Kefalas, T. Kokkinou and D. Varonos

*Department of Experimental Pharmacology, Athens University School of Medicine, Athens 609 (Greece), 10 February 1982*

*Summary.* Several drugs of various pharmacological classes were tested for antimicrobial activity by the agar diffusion technique and minimal inhibitory concentration (MIC) estimation. Among them diclofenac sodium, haloperidol, meclastine fumarate, chlorpromazine, chlorimipramine and promethazine were the more active.

Interference with bacterial growth by therapeutic drugs, other than antibiotics, has not been thoroughly investigated. Of the main drug classes so far studied, an antibacterial property has been reported for narcotic analgesics[1], local anesthetics[2-4], several hormones[5,6] and some psychotropic agents[7-11]. This study aimed at extending the search for antimicrobial activity among various drug categories.

*Materials and methods.* One or more drugs from each of the main drug categories (classified according to their action on the body systems) were studied. For assessment of the antibacterial activity of the drugs the following strains were used: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853). For the disc assay, Petri dishes (90 mm in diameter) were filled with 15 ml of Müller-Hinton agar (Difco). Discs of Whatman's paper No. 3 (5 mm in diameter) impregnated with 0.01 ml of each drug solution were placed on the surface of the agar inoculated with bacterial suspension ($5 \times 10^6$ cells). After incubation of the plates at 37 °C for 24 h the diameter of the inhibition zone was measured. MICs were determined by the agar dilution technique; overnight tryptic soy broth (TSB, Difco) cultures of the test organisms were diluted 1:1000 with fresh TSB and 0.02 ml of this dilution was inoculated onto Müller-Hinton agar plates containing doubling dilutions of the drug. The inoculum level obtained was approximately $2 \times 10^4$ organisms. After overnight incubation at 37 °C, the plates were examined and the MIC recorded as the lowest concentration totally preventing visible growth.

*Results.* Drugs with no detectable antibacterial activity at the concentrations tested are presented in table 1. The mean inhibitory zone and the MIC for the drugs found to possess antimicrobial activity are shown in table 2. Among them the most potent against *Staphylococcus aureus* were diclofenac, haloperidol, meclastine, chlorpromazine, chlorimipramine and promethazine, in decreasing order. Against *E. coli* haloperidol, meclastine, chlorpromazine, chlorimipramine, promazine and promethazine, in the same order, gave the strongest positive results. *Pseudomonas aeruginosa* was very resistant to all drugs tested.

*Discussion.* Antibacterial activity of drugs other than antibiotics may be interesting for 3 reasons. a) Use of drugs possessing antimicrobial properties, prior to obtaining samples for microbiological tests, may be the cause of false negative results. b) As the combined use of several drugs and antibiotics is common, knowledge of the antimicrobial properties of a drug could be of practical importance for anticipating interactions between this drug and antibiotics. c) An investigation of the effects of drugs on single cells may promote the elucidation of the biochemical action of these compounds.

Topical anesthetics have been reported to inhibit bacterial growth[2-4]. Cocaine in a concentration as low as 2.5% was lethal to *Staphylococcus albus, P. aeruginosa* and *Candida albicans*[2]. In our study, approximately the same concentration of lidocaine and procaine was not able to produce an inhibitory zone in the bacteria tested. This difference was expected since the disc assay method is less sensitive in detecting antimicrobial activity, compared with the growth inhibition test used by Kleinfeld and Ellis[2]. The same holds true for morphine and heparin, to which antibacterial activity has been ascribed[1,12]. Synergistic effects of pheno-