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.

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Antibacterial properties of several drug categories

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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 (5×10^6 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 2×10^4 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-

thiazine derivatives (chlorpromazine and perphenazine) on β-lactam antibiotics (ampicillin, carbenicillin, cefazolin) and nalidixic acid group compounds have been found on *E. coli* and *P. aeruginosa* by Yamabe[11]. In his study he found that the MIC of chlorpromazine for *E. coli* NUHJ JC-2 was 125 µg/ml of chlorpromazine reached 62.5 µg/ml in the presence of 1.56 µg/ml nalidixic acid, indicating that the combination effect was additive. Taking into consideration that peak plasma concentrations (PPC) of chlorpromazine have been estimated to be 30–350 ng/ml[13], it is obvious that this combination effect is deprived of any clinical meaning. For diclofenac and meclastine, drugs found in this study to possess strong antibacterial activity, the values of PPC and MIC for *St. aureus* are as follows: diclofenac, PPC = 1.4–3.0 µg/ml[14], MIC = 62 µg/ml; meclastine, PPC = 0.05–0.14 µg/ml[15], MIC = 125 µg/ml. As MIC values of these drugs are closer to PPC values, compared to those of chlorpromazine, one could expect that synergy studies between the former drugs and several antibiotics would be more interesting.

Effect of chlorpromazine and related compounds on microbial cells have been used as probes in investigating their mechanism of action. Intercalation of chlorpromazine into duplex DNA[7], inhibition by this drug of a variety of enzymes[11] and changes in the permeability of bacterial cell walls[10] have been studied in this way. Bacterial models have also been used in investigating the mode of action of drugs of the morphine series[1]. Drugs like diclofenac sodium, a non-steroid antiinflammatory drug, haloperidol, a butyrophenone used as major antipsychotic and meclastine fumarate, a non-phenothiazine antihistamine, whose antibacterial properties were found to be worthwhile nothing in the present study, should be further investigated.

Table 1. Drugs with no detectable antibacterial activity

Drug	Amount per disk	Drug	Amount per disk	Drug	Amount per disk
Adrenalin	5 µg	Hydroergotoxine meth/sulf.	3 µg	Procaine hydrochloride	200 µg
Amiodarone	500 µg	Insulin	0.4 IU	Progesterone	250 µg
Atropine sulfate	10 µg	Lanatoside C	2 µg	Prostigmine bromide	5 µg
Benzonatate	50 µg	Lidocaine	200 µg	Reserpine	10 µg
Camylofine dihydrochlor.	250 µg	Lysine acetylsalicylate	1 mg	Scopolamine brom/butyl.	200 µg
Chlordiazepoxide	200 µg	Mephedermine	1.5 µg	Silomat	100 µg
Chymothrypsin	50 µg	Metaproterenol	20 µg	Strychnine sulfate	10 µg
Cimetidine	1 mg	Metoclopramide	50 µg	Succinylcholine chlor.	250 µg
Clonidine	1.5 µg	Morphine hydrochloride	200 µg	Sulpiride	500 µg
Dexamethasone	50 µg	Naftidrofuryl	100 µg	Synephrine	600 µg
Digoxin	2.5 µg	Nikethamide	2.5 µg	Testosterone propionate	250 µg
Dipyridamole	50 µg	Noradrenalin	20 µg	Theophylline	2.5 mg
Estriol succinate	100 µg	Oxytocin	0.03 IU	Thiocolchicoside	20 µg
Ethylphenylephrine	100 µg	Pancuronium bromide	20 µg	Tranexamic acid	500 µg
Furosemide	100 µg	Phenobarbital sodium	2 mg	Vasopressin	0.05 IU
Gonadotropin hum. chor.	5 IU	Piracetam	2 µg	Verapamil	25 µg
Heparin	50 IU	Prednisolone	250 µg	Vincamine	50 µg
Hexoprenalin	25 µg	Procainamide hydrochl.	1 mg	Xanthinol nicotinate	1.5 mg

Table 2. Inhibitory zones and MICs of the drugs found to have antimicrobial activity

Drug	Concentration (µg/0.01 ml)	*Staphylococcus aureus* Inhibitory zone (mm)*	*Staphylococcus aureus* MIC (µg/ml)	*Escherichia coli* Inhibitory zone (mm)*	*Escherichia coli* MIC (µg/ml)	*Pseudomonas aeruginosa* Inhibitory zone (mm)*	*Pseudomonas aeruginosa* MIC (µg/ml)
Chlorimipramine	125	15.83 ± 0.31	312	13.33 ± 0.33	312	7.17 ± 0.17	> 1250
Caffeine	2500	15.17 ± 0.31	12500	20.50 ± 0.34	6250	9.00 ± 0.37	25000
Chlorpromazine	50	15.83 ± 0.31	250	13.83 ± 0.17	250	0	> 2500
Dextropropoxyphene	375	10.33 ± 0.80	470	0	3750	0	> 3750
Diazepam	50	10.50 ± 0.22	1000	7.33 ± 0.21	1000	7.17 ± 0.17	1000
Diclofenac sodium	250	19.67 ± 0.21	62	7.50 ± 0.43	500	7.50 ± 0.22	500
Dimenhydrinate	500	10.33 ± 0.56	2500	9.50 ± 0.34	2500	8.83 ± 0.31	2500
Dipyrone	5000	9.67 ± 0.33	25000	8.83 ± 0.40	50000	0	> 50000
Haloperidol	50	13.00 ± 0.58	90	8.50 ± 0.22	180	6.67 ± 0.21	720
Imipramine	125	12.33 ± 0.49	1250	11.83 ± 0.31	625	7.17 ± 0.17	> 1250
Meclastine fumarate	10	7.33 ± 0.33	125	6.67 ± 0.21	250	6.83 ± 0.40	500
Meclofenoxate	250	6.83 ± 0.48	890	7.67 ± 0.33	3560	6.83 ± 0.48	> 3560
Methotrimeprazine	250	14.67 ± 0.21	875	0	> 2500	0	> 2500
Orphenadrine	150	8.67 ± 0.42	3000	11.00 ± 0.37	750	0	> 3000
Papaverine hydrochloride	400	8.67 ± 0.33	> 4000	0	> 4000	0	> 4000
Pentazocine	300	9.17 ± 0.40	1500	8.17 ± 0.31	1500	6.67 ± 0.33	> 3000
Perazine	250	16.50 ± 0.22	875	11.17 ± 0.40	875	0	> 3500
Pethidine	500	0	> 5000	12.17 ± 1.25	5000	0	> 5000
Phenylbutazone	2000	16.50 ± 0.22	1250	7.33 ± 0.21	2500	6.33 ± 0.21	> 20000
Phenyramidol	2650	22.00 ± 0.86	6665	24.17 ± 0.31	3332	13.83 ± 0.83	3332
Promazine	500	19.00 ± 0.26	625	20.17 ± 0.31	312	11.33 ± 0.42	2500
Promethazine	250	15.50 ± 0.43	312	17.83 ± 0.17	312	8.33 ± 0.42	> 2500
Propantheline bromide	300	16.17 ± 0.31	750	6.67 ± 0.56	3000	0	> 3000
Salicylate sodium	1000	0	> 15000	8.83 ± 0.87	7142	0	> 15000

*Mean ± SE; n = 7.

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Conformation and local anesthetic activity of carbanilates

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Summary. The influence of spatial configuration on intensity, onset, and duration of anesthetic effect has been observed in some carbanilate local anesthetics of fixed conformation. Synthesis of the carbanilates is described.

One approach to gain insight into membrane local anesthetic 'receptor' configurations is by fitting a variety of isomeric drugs to a putative receptor[1]. A number of position isomers and stereoisomers[2,3] as well as optical isomers[4] has already been prepared and pharmacologically tested in the group of pentacaine derivatives[2,5,6] which belongs to the group of carbanilate local anesthetics. The anesthetic activity of these compounds can be related to some of their physicochemical properties[7]. Boots and Boots[8] and Borne et al.[9] called attention to conformational aspects in norbornyl and azabicyclooctyl esters with local anesthetic activity, respectively. The purpose of the present study was to analyze whether isomers of pentacaine with rigid conformation differ in the local anesthetic activity.

A group of diastereoisomeric pentacaine derivatives conformationally firmly fixed by tertiary butyl group was prepared according to the previously described method[10-12]. By Clarke-Eschweiler methylation, dimethylaminoderivatives were prepared from all the 4 possible vicinal aminoalcohols with subsequent addition on 2-hexyloxyphenyl isocyanate[13]. The resulting 2-hexyloxycarbanilates are shown in the table. Structures of the substances were confirmed by IR-spectroscopy ($\nu_{c=o}$ 1730 cm^{-1}, 4 mg in 400 mg KBr (table), SPECORD IR 75), mass spectroscopy (200 °C, 12 and 75 eV, JEOL IMS-100), GC (aminoalcohols only, CHROM-4) and TLC (ethanol:water:ammonia = 4:3.5:0.1).

Pharmacological effects of the drugs were studied on rat sciatic nerve using a modified method of Paterson and Hamilton[14] and on guinea-pig ileum in vitro[15]. Changes in the size of the compound action potential in isolated sciatic nerve induced by the drugs were measured. To minimize the influence of perineurium as a diffusion barrier, the nerve preparations were longitudinally split into 2 bundles. The local anesthetic effect of the isomers in the concentration 1×10^{-3} moles/l was not identical. Following 120 min of application, the isomer No.3 inhibited the compound action potential almost completely, and isomer No.1, by approximately 30% (fig. 1, A). The other 2 isomers possessed intermediate potency. The differences were statistically highly significant ($p < 0.001$). The identical sequence of drugs has been found in quantities characterizing the dynamics of the local anesthetic effect, namely in the rate of action potential inhibition during the application

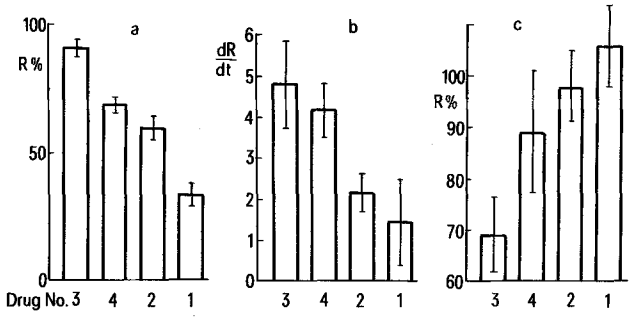

Figure 1. Decrease in compound action potential amplitude in isolated rat sciatic nerve (A), rate of action potential inhibition in the 1st 5 min of drug action (B) (R, reduction of action potential in percent of the effect in the 120th min of application, t, time in min), and inhibition persisting even following 60 min of drug wash-out expressed in percent of the maximal effect (C) induced by the 4 diastereoisomers (see table). Means ± SEM are shown.

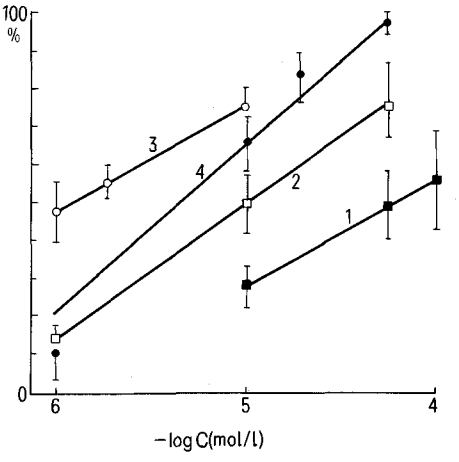

Figure 2. Dependence of inhibition of guinea-pig ileum twitches induced by transmural electrical stimulation on concentration (C) of the 4 diastereoisomers (see table).