Physical and pharmacological data for compounds **Ic**, **Id**, **IIc** and **IId**

Compound	Derivative	Melting point (°C)	$[\alpha]_D^{22}$*	In vivo dose (μmoles/kg) in mice required to produce oxotremorine blockade**	Mydriatic activity relative to atropine
(R)-(−)-Ic	Sesquioxalate	85–86	−22.9	56.0	0.06
(S)-(+)-Ic	Sesquioxalate	86–87	+22.9	2.6	0.07
(±)-Ic	Sesquioxalate	74–75		5.0	0.05
(R)-(+)-Id	Sesquioxalate	82–83	+ 1.2	6.5	0.024
(S)-(−)-Id	Sesquioxalate	82–83	− 1.4	5.8	0.017
(±)-Id	Sesquioxalate	91.5–92.5		5.2	0.024
(R)-(−)-IIc	Base	53.5–54.5	−58.4	17.4	0.024
(S)-(+)-IIc	Base	54.5–55.5	+59.9	8.3	0.027
(±)-IIc	Base	52–53		13.5	0.022
(R)-(+)-IId	Oxalate	104.5–106	+ 0.8	20.0	0.001
(S)-(−)-IId	Oxalate	105–106	− 0.8	17.2	Miosis
(±)-IId	Oxalate	108–110		17.9	Inactive
Atropine				2.8	1

*All rotations were measured in ethanol (c 1.0–1.7). **Dose of test compound required to double the dose of oxotremorine inducing a grade 2 tremor in 50% of the mice.

It is evident from the table that introduction of a methyl group in the pyrrolidine ring of oxotremorine **(Ia)** affords compounds which are antagonists to oxotremorine. The same substitution in the oxotremorine antagonist **IIa** enhances the antagonistic activity. Only the enantiomers substituted in the 2-position show stereoselectivity, the S-isomers being the most active. However, this selectivity is less pronounced than that of the enantiomers of the compounds with the chiral centre in the 1-position of the butynyl chain **(Ib, IIb)**[3]. These results are somewhat similar to those obtained with parasympatholytic agents of the amino ester type, whose activity is critically dependent on the configuration of a chiral centre in the acyl moiety but independent of the configuration of the amino alcohol part of the molecule[12]. The compounds in the table have weak mydriatic activity and no stereoselectivity can be observed.

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Renal accumulation of amikacin, tobramycin and gentamycin in the rat

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Summary. Free and total concentrations of amikacin, tobramycin and gentamycin were measured separately in the rat kidney after equal weight by weight doses. The accumulation of aminoglycosides followed the order amikacin < tobramycin < gentamycin. The ratio between free and total aminoglycosides was similar (about 0.6) in all 3 aminoglycosides and independent on the length of administration.

The aminoglycoside antibiotics are potentially nephrotoxic agents[1,2]. The renal accumulation of many aminoglycosides has been compared using mainly the rat as experimental animal[3–5]. However, the renal accumulation of amikacin, a new aminoglycoside derivative, in comparison with tobramycin and gentamycin, has not been established. In the present study, the renal accumulation of amikacin, tobramycin and gentamycin were investigated in young female rats. Since aminoglycosides become partially bound to tissue macromolecules and thus inactivated[6], free and total aminoglycoside concentrations were measured separately.

Material and methods. 1-month-old female Sprague-Dawley rats (90–110 g) were selected for the experiment. The rats were maintained on a standard diet (R3/Astra-Ewos) and tap water ad libitum. Amikacin (Bristol Laboratories), tobramycin (Nebcina®, Lilly) and gentamycin (Garamycin®, Schering) were administered at the dose of 40 mg/kg s.c. once a day. The treatment lasted 1, 5 or 12 days. The

		Concentration of aminoglycosides (μg/g) Free	Total	Free/Total	Degree of histological lesions
1 Dose	Amikacin	10±2	16±4	0.64±0.03	0
	Tobramycin	30±1[b]	57±16 ns	0.60±0.13	0
	Gentamycin	65±4[c,e]	105±8[b] NS	0.63±0.08	0
5 Doses	Amikacin	49±2	75±10	0.68±0.10	0
	Tobramycin	124±12[b]	195±30[a]	0.65±0.07	0
	Gentamycin	187±22[b] NS	387±18[c,d]	0.49±0.08	0.3±0.3
12 Doses	Amikacin	74±5	148±9	0.50±0.04	0
	Tobramycin	174±45 ns	288±45[a]	0.59±0.08	0
	Gentamycin	269±40[b] NS	532±101[a] NS	0.53±0.07	1.7±0.3

The concentrations and ratios of free and total aminoglycosides and the degree of histological lesions in the rat kidney (mean ± SE, n = 3). Significance of difference from amikacin is shown by ns = not significant, [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$. Significance of difference between tobramycin and gentamycin, is shown by NS = not significant, [d] $p < 0.01$, [e] $p < 0.001$

animals were killed 24 h after the last drug injection. 1 kidney was rapidly removed and as similar as possible latitudinal slices were cut from the middle part. The slices were homogenized in phosphate buffer (0.1 M, pH 8) with a Potter-Elvehjem homogenizer. 2 equal homogenates were prepared from each kidney, one for the determination of free, another for the determination of total aminoglycosides. The aminoglycosides were determined microbiologically using *Bacillus subtilis* as the marker organism, free and total separately as described by Kornguth and Kunin.

Kidney samples were taken from each kidney for histological examination. They were fixed in buffered 10% formalin, embedded in paraffin, sectioned at 7 μm and stained with hematoxylin eosin. The degree of histological lesions was estimated as follows. 0 = no changes; 1 = small eosinophilic granules in the cytoplasm of the epithelial cells of proximal convoluted tubules, no changes in the nuclei and basement membranes; 2 = necrotic changes in some of the proximal convoluted tubules. Statistical analyses were performed by Student's t-test.

Results and discussion. The results are presented in the table. The concentrations of free and total aminoglycosides in the renal tissue followed the order amikacin < tobramycin < gentamycin, when administered in equal weight by weight doses (mg/kg/day). The ratio between free and total aminoglycosides was of the same order independent on the lenght of the administration and on the aminoglycoside used (about 0.6).

Histological lesions in the kidney were observed only in the groups given gentamycin 5 and 12 doses. In these groups the mean concentration of aminoglycoside was also the highest. It is not known with certainty whether or not the renal concentrations of aminoglycosides correlate with the degree of nephrotoxicity[4]. The present work as well as our recent observations indicate that there is a correlation within certain limits of concentration. No histological tissue damage was observed with low concentrations (roughly < 200 μg/g free aminoglycoside in the whole kidney). On the other hand, the extensive tissue damage occurring with high dose levels sets the limit to the amount of aminoglycoside taken up by the kidney. Thus, no correlation between dose and tissue damage is observed here.

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A triple test for screening biological activity of prostacyclin analogues

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Summary. 6,9-Thiaprostacyclin was used as a representative of prostacyclin analogues with the aim to design the most convenient procedure for evaluation of vasodilator, anti-platelet and other biological properties within this new group of potential anti-thrombotic agents.

Recently, a new vasodilator and anti-thrombotic hormone was discovered[1,2], synthetized[3,4] and named prostacyclin (PGI_2). Prostacyclin is supposed to protect blood vessels against thrombosis[2] and against atherosclerosis[5,6]. Prostacyclin molecule has been thrust into the forefront of chemical prostaglandin research[3,4,7]. The main goal is to obtain a stable analogue of the natural unstable hormone.

Any PGI_2 analogue may mimic biological activity of the parent hormone, but it also may have biological activity of prostaglandins (PGs) or thromboxane A_2 (TXA_2) which are the other members of arachidonic acid cascade[8]. A method here proposed differentiates between these activities on the basis of reaction of vascular smooth muscle, inhibition of blood platelet aggregation in vitro and reversal of platelet aggregation in vivo.

In this study, we used prostacyclin (PGI_2) and 6,9-thiaprostacyclin (TP), both of which were synthetized by Nicolaou et al.[7]. The stock solutions were prepared in 99% ethanol