J. Pharm. Pharmacol. 1988, 40: 657-659
Communicated February 24, 1988

A comparison of the effects of oxodipine and nifedipine on rat vas deferens

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Abstract—The effects of oxodipine, a new dihydropyridine, were studied and compared with those of nifedipine in the complete vasa deferentia and in the prostatic and epididymal halves of the rat vas deferens. Oxodipine and nifedipine, 10^{-9}–10^{-6}M, produced a dose-dependent inhibition of the contractile responses induced by single pulse stimulation, noradrenaline (3×10^{-5}M) and high-K (50 mM). The inhibitory effects of oxodipine were significantly reduced in high Ca media. From these experiments it is concluded that oxodipine, like nifedipine, produced a similar and potent inhibitory effect of the contractile responses induced in the rat vas deferens.

Ca channel blockers, such as nifedipine, are powerful smooth muscle relaxants, an effect which has been explained by means of the inhibition of Ca entry through voltage-dependent Ca channels in the muscle membrane (Fleckenstein 1983; Nayler & Dillon 1986). In fact nifedipine antagonizes the contractile response induced by noradrenaline and KCl as well as those induced by single pulses of field stimulation (French & Scott 1981; Hay & Wadsworth 1983).

Oxodipine, [4-(2,3-methylen-dioxyphenyl)-1,4-dihydro-2,6-dimethyl-3-carboxyethyl-5-carboxymethyl-pyridine], is a new dihydropyridine derivative (Fig. 1) which exerts potent vasodilator effects in isolated vascular smooth muscle fibres (Marin et al 1985). However, its effects have not been previously studied in the isolated vas deferens. So, the present study was undertaken to study and compare the effects of oxodipine with those of nifedipine in the rat isolated vas deferens.

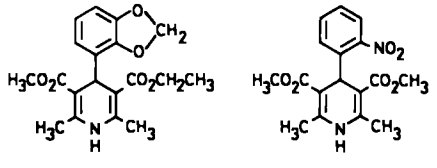

FIG. 1. Chemical structure of oxodipine and nifedipine.

Materials and methods

Vasa deferentia were removed from mature male Wistar rats (250–300 g). Tissues were suspended under 0·5 g tension in a 20 mL organ bath containing Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·18, $NaHCO_3$ 25, KH_2PO_4 1·2 and glucose 11. The solution was continuously bubbled with 95% O_2–5% CO_2, resulting in a pH of approximately 7·4. Contractile responses were recorded isometrically by means of Grass FT 03 force-displacement transducers.

Tissues were stimulated using a Grass SD 9 stimulator delivering a single square wave pulse (1 ms duration, 100 V) every 4·5 s through two parallel platinum electrodes. Twitch contractions were allowed to equilibrate (about 30 min). The nifedipine and oxodipine were added to the bath cumulatively with each concentration being left in contact until its effect reached a plateau (about 15 min). Contractions were also obtained with noradrenaline (NA), 3×10^{-5}M, and KCl 50 mM (contact period approximately 1 min) repeated every 20 min. When two successive contractions of similar amplitude were obtained oxodipine or nifedipine were added and allowed to equilibrate for 15 min before repeated challenge with noradrenaline or KCl.

The drugs used were: oxodipine (IQB S.A.), nifedipine (Bayer) and noradrenaline hydrochloride (Sigma). Oxodipine and nifedipine were dissolved in ethanol and thereafter further dilutions were made in Krebs solution. When nifedipine was used, the bath and solutions were protected from light. Results are given as mean ± s.e.m. Statistical analysis of the data was made with Student's *t*-test and the 0·05 level of probability was regarded as significant.

Results

Contraction induced by single pulse stimulation. Oxodipine and nifedipine 5×10^{-8}–8×10^{-7}M produced a dose-dependent inhibition of the twitch responses induced in the vasa deferentia (Fig 2). The effect of nifedipine was more marked but not statistically different ($P > 0.05$) from that of oxodipine. The inhibitory effects of both drugs were rapidly reversed after washing with drug-free Krebs solution.

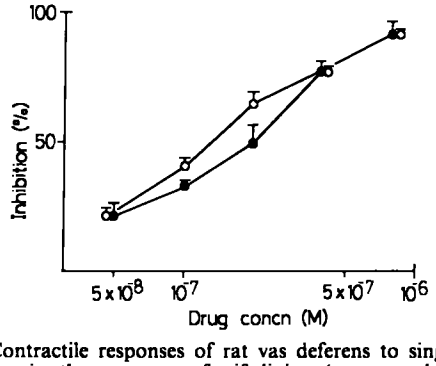

FIG. 2. Contractile responses of rat vas deferens to single pulse stimulation in the presence of nifedipine (open symbols) and oxodipine (closed symbols). Ordinate: inhibition of the maximal control response (%). Abscissa: drug concentration (M). Each point is the mean of 9 tissues; vertical bars show s.e. mean.

Contractions induced by NA and KCl. In a first group of experiments the effects of oxodipine and nifedipine were studied on the contractions induced by 3×10^{-5}M NA and 50 mM KCl. Oxodipine and nifedipine dose-dependently reduced the contractile responses induced by NA (Table 1). At concentrations between 2.5×10^{-8} and 10^{-7}M the inhibitory effect of nifedipine was significantly higher than that of oxodipine ($P < 0.01$). However, at the highest concentration tested, 2×10^{-7}M, there

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Table 1. Effect of nifedipine and oxodipine on the contractile responses induced by NA, 3×10^{-5}M in rat vasa deferentia. Steady-state responses are expressed as a percentage of the pre-drug control values (mean ± s.e.m., n = 9).

Drug Concn (M)	Inhibition (%) Nifedipine	Oxodipine
2.5×10^{-8}	29·8 ± 6·5	8·2 ± 4·6*
5×10^{-8}	54·1 ± 2·8	22·1 ± 2·6*
1×10^{-7}	64·3 ± 4·7	44·3 ± 4·3*
2×10^{-7}	67·8 ± 2·8	67·5 ± 2·4

* $P < 0.01$.

was no significant difference in the inhibitory effect produced by oxodipine and nifedipine ($P > 0.05$). Oxodipine and nifedipine (10^{-9}–10^{-7}M) also produced a dose-dependent decrease in the contractile responses induced by 50 mM KCl (Table 2). The

Table 2. Effect of nifedipine and oxodipine on the contractile responses induced by 50 mM KCl in complete and epididymal and prostatic portions of rat vas deferens. Steady-state responses are expressed as a percentage of the pre-drug control values (mean ± s.e.m., n = 9)

Drug Concn (M)	Complete vas deferens	Epididymal portion	Prostatic portion
Nifedipine			
10^{-9}	33·7 ± 8·7	20·2 ± 2·6	25·8 ± 2·1
5×10^{-9}	40·8 ± 7·1		
10^{-8}	45·2 ± 7·7	39·8 ± 4·5	55·3 ± 3·5
2.5×10^{-8}	55·4 ± 3·5		
10^{-7}	82·3 ± 5·1	77·4 ± 4·1	80·7 ± 2·3
Oxodipine			
10^{-9}	16·5 ± 13·0	40·1 ± 2·9	42·3 ± 3·3
5×10^{-9}	17·4 ± 7·1		
10^{-8}	22·4 ± 6·7	46·2 ± 2·7	54·8 ± 2·6
5×10^{-8}	59·1 ± 4·2		
10^{-7}	73·4 ± 5·3	80·3 ± 3·8	58·0 ± 3·1

inhibitory effects induced by nifedipine, 5×10^{-9} and 10^{-8}M, were significantly higher than that induced by oxodipine ($P < 0.01$), whereas at higher concentrations no significant differences were found between nifedipine and oxodipine. The effects of oxodipine and nifedipine on contractile responses induced by NA and high K were readily reversed by washout of the drugs from the organ bath.

The effects of oxodipine were antagonized when the Ca concentration of the bathing media was increased from 2·7 to 5·4 mM. For these experiments we selected the concentration of oxodipine required to reduce by almost 50% the contractile responses induced by single pulse stimulation (2×10^{-7}M), KCl (2.5×10^{-8}M) and NA (10^{-7}M). As is shown in Fig 3, oxodipine inhibited to a lesser extent the maximum height of the contractile responses induced by single pulse stimulation ($P < 0.01$), NA ($P < 0.01$) and high K ($P < 0.05$) in 5·4 mM Ca than in normal Krebs solution.

Discussion

The results of this paper show that oxodipine, a new dihydropyridine, produced in the rat isolated vas deferens a dose-dependent inhibition of the contractile responses induced by KCl, NA or single pulse field stimulation. Moreover, this inhibition was antagonized by increasing the Ca concentration in the bathing media. Similar effects were found with nifedipine, the best known dihydropyridine, in this paper and by others (Hay & Wadsworth 1983; French & Scott 1981).

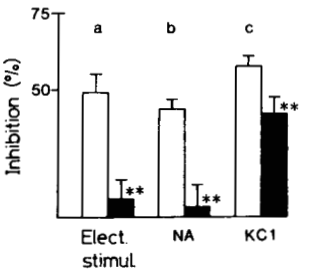

FIG. 3. Effects of oxodipine on the contractile responses induced by (a) single pulse stimulation and (b) noradrenaline, 3×10^{-5}M, and by (c) high-K (50 mM) in rat vas deferens incubated in Krebs solution containing 2·7 mM Ca (open columns) or 5·4 mM Ca (solid columns). Values are the mean of at least 11 experiments; vertical bars show s.e. mean. * $P < 0.05$. ** $P < 0.01$.

Contractions induced by high-K or single pulse stimulation have been attributed to the influx of Ca through voltage-operated channels (Bolton 1979) as well as to the release of neurotransmitters from nerves following depolarization (Hay & Wadsworth 1980; Ceña et al 1985). Thus, the inhibitory effects of oxodipine and nifedipine could be explained by a blockade of Ca influx through voltage- and receptor-operated channels and/or to the reduction of neurotransmitter release. However, it is unlikely that the inhibition of high K-induced contractions can be attributed to a decrease of NA release since nifedipine at the same range of concentrations which inhibited the contractility caused release of [^{3}H]NA in vas deferens of guinea-pig (Zsoter & Wolchinsky 1986). As previously described for nifedipine (Hay & Wadsworth 1983), about 20% of the high K-induced contractions are resistant to both nifedipine and oxodipine. This component can be blocked by verapamil which suggests that there are probably two types of Ca activation mechanism, one which is blocked by dihydropyridines but both by verapamil (Hay & Wadsworth 1980).

The responses induced by NA in rat vas deferens were readily inhibited to a similar extent by oxodipine and nifedipine. NA-induced contractions have been attributed to Ca entry through receptor-operated channels (Bolton 1979). However, NA also depolarized the vas deferens (Magaribuchi et al 1971), and membrane depolarization mediates that part of the response to NA which is permissive to oxodipine and nifedipine. Another possible explanation is the existence of different receptor-operated channels, one of which is insensitive to dihydropyridines.

Fleckenstein (1983) proposed that elevation of Ca concentration could neutralize the effects of Ca channel blockers. In fact, in rat vas deferens the effects of nifedipine were antagonized when the Ca concentration in the bathing media was increased (French & Scott 1981). As expected, in the present experiments the inhibitory effects of oxodipine on the contractions induced by single pulse stimulation, high K and NA were also antagonized by increasing the Ca concentration to 5·4 mM.

In conclusion, the present results demonstrated that oxodipine, a new dihydropyridine, like nifedipine, exhibited potent inhibitory effects of the contractile responses induced by high-K, NA and single pulse stimulation in the rat vas deferens.

The authors express their gratitude to Dr A. Galiano of IQB for the generous supply of oxodipine. This work was supported by a CAICYT Grant (2074/83).

References

Bolton, T. B. (1979) Mechanisms of action of transmitters and other substances on smooth muscle. Physiol. Rev. 3: 606–718

Ceña, V., Garcia, A. G., Khoyi, M. A., Salaices, M., Sánchez-García, P. (1985) Effect of the dihydropyridine Bay K 8644 on the release of [^{3}H]-noradrenaline from the rat isolated vas deferens. Br. J. Pharmacol. 85: 691–696

Fleckenstein, A. (1983) In: Calcium antagonism in heart and smooth muscle. Experimental facts and therapeutic prospects. John Wiley & Sons. New York

French, A. M., Scott, N. C. (1981) A comparison of the effects of nifedipine and verapamil on rat vas deferens. Br. J. Pharmacol. 73: 321–323

Hay, D. W. P., Wadsworth, R. M. (1980) Potassium contractures in the rat isolated vas deferens: the role of noradrenaline release and of extracellular calcium. Ibid. 72: 143P–144P

Hay, D. W. P., Wadsworth, R. M. (1983) The effects of calcium channel inhibitors on twitches and noradrenaline contractions of the rat bisected vas deferens. Eur. J. Pharmacol. 87: 367–378

Magaribuchi, T., Ito, Y., Kuriyama, H. (1971) Effects of catecholamines on guinea-pig vas deferens in various ionic environments. Jap. J. Physiol. 21: 691–696

Marín, J., Salaices, M., Tamargo, J., Tejerina, T. (1985) Effects of oxodipine on vascular smooth muscle fibres. Br. J. Pharmacol. 86: 500P

Nayler, W. G., Dillon, J. S. (1986) Calcium antagonists and their mode of action: a historical overview. Br. J. Clin. Pharmacol. 21: 97S

Zsoter, T. T., Wolchinsky, C. (1986) The effect of nifedipine on norepinephrine release. Arch. Int. Pharmacodyn. Ther. 280: 32–44

J. Pharm. Pharmacol. 1988, 40: 659–661
Communicated November 24, 1987

The influence of concentration of two salicylate derivatives on rectal insulin absorption enhancement

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Abstract—3,5-Diiodosalicylate sodium (DIS), a highly lipophilic salicylate, was evaluated against 5-methoxysalicylate sodium (MS) as a potential adjuvant absorption promoter for rectal insulin delivery. Comparative blood glucose measurements were made using the two adjuvants under identical conditions as promoters of rectal insulin absorption in rats. Concentrations of DIS greater than and including 0·1 M produced an unexpected, progressive decrease in adjuvant activity as determined by a decline in observed hypoglycaemic response. This was not due to formation of an insulin-DIS complex. The adjuvant MS produced a classical, sigmoidal log-dose response curve. Possible reasons for the occurrence of the DIS optimum phenomenon are discussed as well as are the observed differences in adjuvant potency of these agents in a propylene glycol-containing vehicle.

Salicylate analogues can enhance the absorption of insulin through the gastrointestinal epithelial mucosa by a reversible and apparently non-damaging mechanism (Nishihata et al 1981, 1983; Nakanishi et al 1984).

Structure-activity studies on the effects of salicylate analogues on membrane ion permeability demonstrated that their relative potencies are correlated with their octanol:water partition coefficients and pK_a values (Levitan & Barker 1972).

We have sought to determine if the same factors governing the relative membrane ionic permeability activity of salicylates could be related to their adjuvant potency as absorption promoters for the rectal delivery of insulin.

Materials and methods

Chemicals. Insulin human injection, USP (Eli Lilly, Indianapolis, IN) was used. The 5-methoxy- and 3,5-diiodo-analogues of salicylic acid were obtained from Aldrich Chemical Company, Milwaukee, WI, and converted to the sodium salts by titration with sodium hydroxide in absolute ethanol (Levitan & Barker 1972).

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Preparation of microenema solutions. Microenema solutions were freshly prepared by dissolving the appropriate amount of adjuvant in a vehicle consisting of 0·2 M phosphate buffer (pH 5) containing 50% propylene glycol, which was necessary to solubilize DIS. Insulin was then added to a final concentration of 6 iu mL^{-1} of solution.

Insulin absorption studies. Male Sprague-Dawley rats (200–300 g) from Taconic Farms (Germantown, NY) were fasted for 20 h before experiments for which they were anaesthetized with pentobarbitone sodium (50 mg kg^{-1}). The right jugular vein was cannulated with 0·020-inch i.d. silastic tubing (Dow-Corning Corporation, Midland, MI) inserted to about 3·2 cm past the clavicle and secured with 3–0 silk thread; the cannula was flushed with heparin in 0·9% NaCl (5 units mL^{-1}) (Grasela & Rocci 1984). The animals were placed ventral-side down and microenemas, 1 mL kg^{-1} administered at a depth of 1 cm from the anus which was ligated with thread (Nishihata et al 1984). Serial blood samples (100 μL) were drawn immediately before and at 15, 30, 60, 90, and 120 min following the microenema. Blood was not replaced. Blood samples were immediately transferred to 1·5 mL polypropylene tubes—heparinized by adding 20 μL of a 20 units mL^{-1} solution to the tubes and permitting it to evaporate. All samples were stored over ice until assay on the same day of the experiment. Immediately before assay, blood samples were warmed to 25°C.

Glucose determinations were made on 20 μL amounts of blood using a commercially available kit (Accu-Check II, Boehringer Mannheim Diagnostics Division, Indianapolis, IN).

Insulin-DIS binding study. Binding of DIS by insulin to form a less bioavailable complex could possibly be responsible for the optimum phenomenon observed. We therefore evaluated the affinity of the insulin molecule for DIS by an ultrafiltration technique.

A 2·5 mL sample of a solution of biosynthetic human insulin (Eli Lilly, Indianapolis, IN) at 1 mg mL^{-1} and DIS 1·0 mM was prepared in a vehicle of 0·2 M phosphate buffer (pH 5) and 50% propylene glycol. A control solution similarly prepared con-