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Effects of derivatives of NGP 1-01, a putative calcium channel antagonist, on electrically stimulated guinea-pig papillary muscle

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In earlier work we have reported calcium antagonistic properties for the polycyclic compound NGP 1-01. We now have derivatized NGP 1-01 by side-chain substitution to obtain ten novel aromatic and aliphatic imino-keto and amino-ether compounds. Electrophysiological tests were conducted on these compounds using isolated guinea-pig papillary muscle preparations to record calcium-mediated (slow) action potentials (APs). The lipophilicities of the compounds, expressed as chromatographically determined R_M values, were measured and the molecular surface areas calculated. Several derivatives showed increased activity compared with NGP 1-01. All compounds with aromatic side-chains (benzyl, phenethyl, phenylpropyl) were active (concentrations required for complete suppression of the AP varied between 1×10^{-5} M and 5×10^{-5} M) and compounds with shorter (methyl, butyl) aliphatic side-chains were inactive whilst activity increased dramatically in those compounds with octyl side-chains. Lipophilicity and calculated molecular volumes correlated linearly and bulkier, more lipophilic molecules had increasing activities in the electrophysiological assay. We therefore conclude that bulky substituents on the nitrogen atom increase calcium antagonistic activity in this series of compounds.

1. Introduction

Numerous reports on several interesting novel organic compounds with calcium channel antagonist properties have been published in recent years (for reviews see [1] and [2]). Of these, one of the most interesting is the polycyclic amino compound NGP 1-01 [3-11], mainly due to the fact that this compound is structurally unrelated to any of the known classes of Ca^{2+} channel antagonists. In an early report [3], the structure of NGP 1-01 was reported as 3-hydroxy-4-benzyl-4-azahexacyclo[5.4.1.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$.$0^{8,11}$]-dodecane based on structural assignments as published by Sasaki et al. [12, 13]. We have subsequently reinvestigated [4] the structure of NGP 1-01 after discovering certain discrepancies in spectral properties between our own compounds and those prepared by the group of Sasaki [12, 13]. Simultaneous to our own findings, Marchand and his group [14] reported identical results, confirming our assignment of the structure of NGP 1-01 as 8-benzylamino-8,11-oxapentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecane (**4a**). Our own crystallographic investigation yielded the diagram of NGP 1-01 as depicted in Fig. 1 [4]. It is interesting to note that sodium cyanoborohydride reduction of the imino intermediates **3** yields the azahexacyclic compounds **5** as originally assigned to NGP 1-01 [14]. Compounds with this general structure are currently being synthesized and tested and results will be reported soon.

The current study examines the inhibitory effects of derivatives of NGP 1-01 on the calcium-dependent processes of excitation and contraction in heart cells. Whole cell experiments provided easy access to qualititave evaluation of structure-activity as well as dose-response relations [15]. A series of polycyclic amino compounds related to **4a** was synthesized by side-chain substitution to optimize the calcium antagonistic activity and attempt to derive some structure-activity relationships within this series. Differences in activity between aromatic and aliphatically substituted derivatives were also studied.

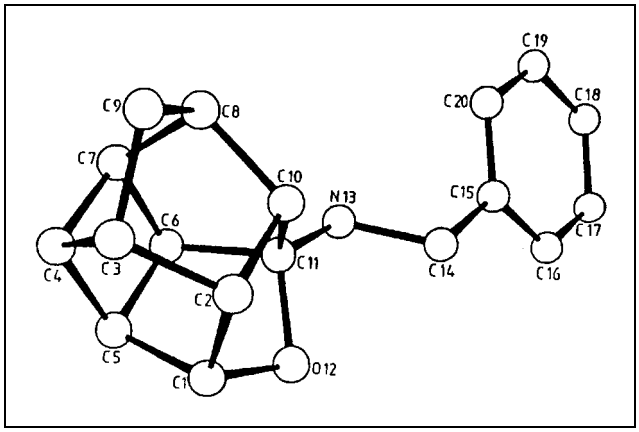


Fig. 1: ORTEP perspective drawing of NGP 1-01 (**4a**) as determined by X-ray crystallography [4].

2. Investigations, results and discussion

2.1. Syntheses of the test compounds

Eleven test compounds were prepared according to the following general scheme using the well-established methods described by Sasaki et al. [12, 13].

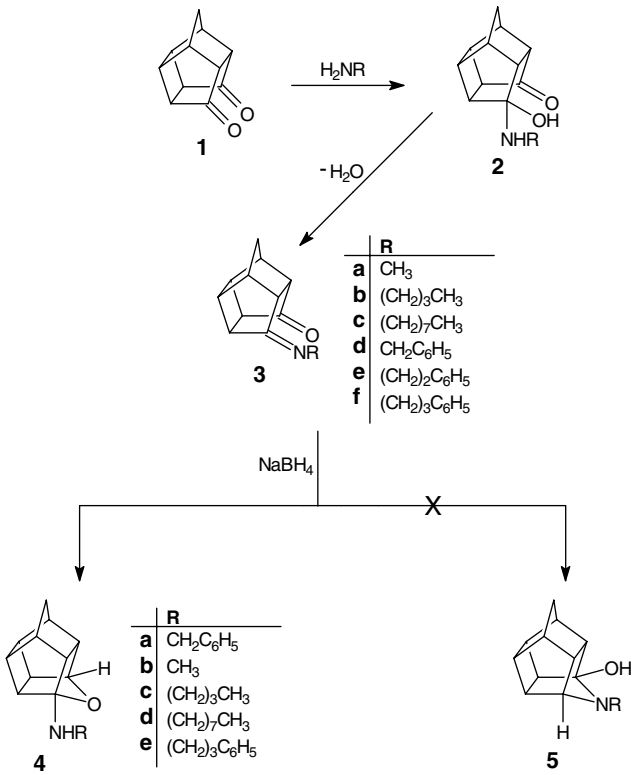
The structures and some physical data are provided in the Scheme and in Table 1. Both the imino compounds **3a–f**

Table 1: Physical data of six imino-keto (3a–f) and five amino-ether (4a–e) derivatives of NGP 1-01

Compd.[a]	R	νC=O cm^{-1} [b]	M^{+} [c]
4a (NGP 1-01)	$-CH_2C_6H_5$	–	265
3a	$-CH_3$	1720	187
3b	$-(CH_2)_3CH_3$	1740	229
3c	$-(CH_2)_7CH_3$	1740	285
3d	$-CH_2C_6H_5$	1710	263
3e	$-(CH_2)_2C_6H_5$	1740	277
3f	$-(CH_2)_3C_6H_5$	1745	291
4b	$-CH_3$	–	189
4c	$-(CH_2)_3CH_3$	–	231
4d	$-(CH_2)_7CH_3$	–	287
4e	$-(CH_2)_3C_6H_5$	–	293

[a] Satisfactory elemental analyses obtained: C, ± 0,38; H, ± 0.2
[b] Nicolet 5DX FT-IR spectrophotometer
[c] VG 7070E and DEC 2035 mass spectrometers

Scheme



as well as the ring-cyclized ethers **4a–e** were synthesized and purified. Verapamil was purchased from Sigma (St. Louis, MO, USA, catalog no. V4629).

2.2. Determination of lipophilicities

The lipophilicities of the synthesized compounds were determined as R_M values calculated according to the method of Boyce and Milborrow [16]. The compounds were applied to chromatographic plates (Merck, Inc. RP-18 F_{254S}) and run in a mobile phase of water/acetone in a 40 : 60 ratio. R_M values were derived from the following equation:

$$R_M = \log (1/R_f - 1)$$

Where R_f is the chromatographically determined R_f-value. R_M-values for the 11 test compounds, as well as for verapamil, are listed in Table 2.

2.3. Calculation of molecular surface areas

The molecular surface area can be defined as the surface formed by the centers of water molecules (regarded as spheres) placed in close contact in all possible positions around a solute molecule [17, 18]. We calculated the molecular surface areas of all the molecules (data shown in Table 2) in order to correlate molecular size or "bulkiness" with other physical parameters such as lipophilicity and also with calcium channel blocking activity. Grid molecular surface areas were calculated using the ChemPlus suite in the HyperChem (Version 4.5) molecular modeling software package. Molecular structures were built and minimized in HyperChem using the molecular mechanics program MM+. After structure minimization, the structures were submitted to ChemPlus for calculation of the molecular surface area expressed as $Å^2$.

Table 2: Empirically determined (reversed phase TLC) R_M-values and calculated grid molecular surface areas (in $Å^2$)[a] for compounds 3a–f and 4a–e

Compd.	R_M	Surface area $Å^2$	Compd.	R_M	Surface area $Å^2$
3a	0.3888	365.13	**4a** (NGP 1-01)	0.8256	481.38
3b	0.4771	445.08	**4b**	0.2188	358.14
3c	2.1632	559.64	**4c**	0.7204	455.2
3d	2.1603	489.02	**4d**	1.3685	578.14
3e	1.8613	515.64	**4e**	1.0633	540.6
3f	2.1604	543.6	Verapamil	0.509	811.61

[a] Hyperchem 4.5® Chemplus Module

2.4. Electrophysiology

2.4.1. Data acquisition

Electrophysiological studies were conducted on guinea-pig papillary muscle. Each compound was tested separately on a fresh preparation. Field stimulation – using two parallel silver wires, isolated at the tips and placed on each side of the preparation – was used. Impulses were generated by means of a Grass S48 stimulator, isolated by a Grass SIU5 stimulus isolation unit (1 Hz stimulation frequency; voltage 10% above threshold). Action potentials were recorded via a micro electrode introduced into the cell and coupled via a high impedance pre-amplifier to a Tektronix R5031 double beam digital oscilliscope in an arrangement similar to that described earlier [3].

2.4.2. Superfusion arrangement

The bathing solution (pH 7.4) for calcium mediated action potentials had the following composition ($mmol \cdot l^{-1}$): NaCl 112, KCl 27, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $NaHCO_3$ 11.9, NaH_2PO_4 0.32, glucose 10, gassed with 95% O_2/5% CO_2 and kept at 37 ± 0.1 °C. The solution was pumped through the bath with a flow velocity of 2–3 $ml \cdot min^{-1}$. The perfusion arrangement was such that switching from the drug containing solution to the regular perfusing fluid could be achieved without any significant alteration in either the flow rate or temperature of the solutions [5].

All compounds in the series were tested as racemates (it has been shown [10] that the enantiomers of NGP 1-01 do not differ in their calcium channel activity). Dosages were titrated down to the minimum dosage required to elicit total suppression of the calcium-mediated action potential (active compounds), or to a concentration of 1×10^{-4} M. Compounds showing no activity at this concentration were classified as inactive. The use of the calcium-mediated action potential for the quantitative evaluation of calcium antagonism of substances has been validated by studies where this method was compared directly with recordings of the slow inward current [15, 19, 20], while inhibition of the slow inward current by NGP 1-01-related compounds had also previously been recorded with suction pipettes in isolated guinea-pig ventricular myocytes [5].

Compounds **3a**, **4b**, **4c** (Scheme, Table 1) showed no activity at all. Compound **3b** was able to partially suppress the AP, and then the only at a high concentration of 8×10^{-5} M. All the other compounds were able to suppress the calcium (Ca^{2+}) mediated AP completely at concentrations varying from 1×10^{-4} M to 1×10^{-5} M. Fig. 2 illustrates the effects of two active imino compounds (**3c**, **d**), two active amino-ether derivatives (**4d**, **e**) and the effects of verapamil in comparison with the inactive compound **3a**. Note the similarities both in lipophilicities and mole-

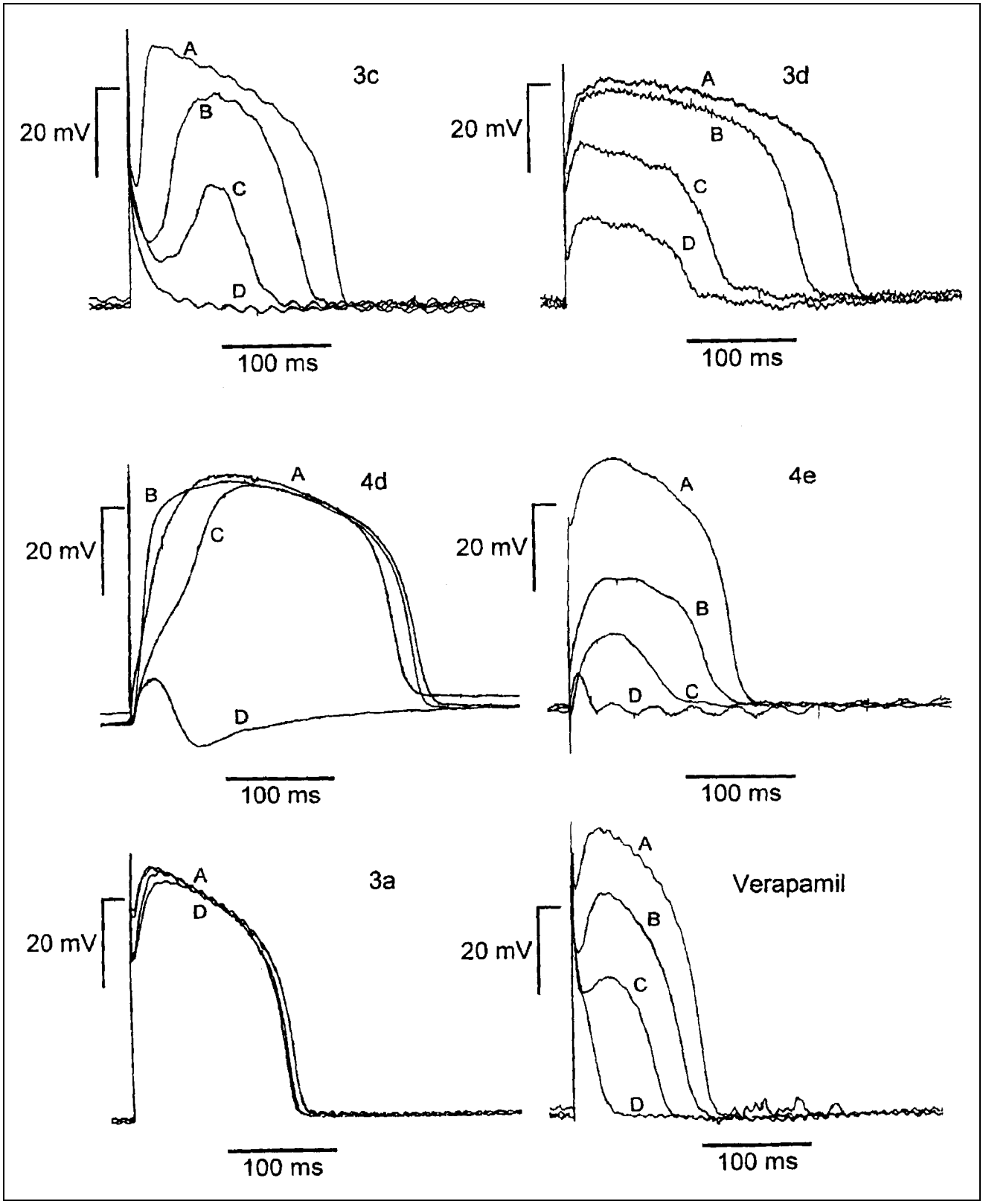


Fig. 2: Calcium mediated action potentials (guinea-pig papillary muscle) under the influence of **3c** (left, top row), **3d** (right, top row), **4d** (left, center row), **4e** (right, center row) (all at concentrations of 5×10^{-5} M), **3a** (left, bottom row) at a concentration of 1×10^{-4} M and verapamil (right, bottom row) at a concentration of 4×10^{-5} M. A = control tracing with no drug. Time allowed to reach D after start of application of drug was 30 min for **3a** (no effect); maximal inhibition (tracing D) for **3d** and **4e** was reached after 20 min (tracings B and C respectively, were obtained after 5 and 10 min for **3d** and after 10 and 15 min for **4e**), 15 min for verapamil (B and C traced after 5 and 10 min, respectively), 10 min for **3c** (B and C after 3 and 6 min, respectively) and 5 min for **4d** (tracings B and C at 1 and 3 min, respectively). For all polycyclic compounds and verapamil the washout times were consistently similar to their incubation times

cular surface area of compounds **3c** and **3d** (Table 2) and also the similarity in electrophysiological effects on the slow AP despite the vast difference in chemical structure, **3c** being a long chain aliphatically substituted imino-ketone, whilst **3d** has an aromatic side chain.

A similar tendency is seen with compounds **4d** and **4e**. However, although the lipophilicities of **3a** and verapamil are very similar, their molecular surface areas differ substantially and compound **3a** had no calcium antagonistic activity, contrasting sharply with verapamil in this respect. The fact that activity in this series of compounds only resided in derivatives with higher lipophilicity and that compounds **3a**, **b** and **4b**, **c** were found to be practically inactive, may indicate that lipophilicity plays a role in the activity of these compounds. Also, it is likely that "bulkiness", as reflected in the molecular surface area, may play an even bigger role. Compounds in this series with smaller molecular surface areas tended to be inactive compared with their "larger" counterparts. Verapamil belongs to the diarylalkylamino group of compounds, which is totally unrelated to the polycyclics described in this paper, possibly having a different molecular mode of action which may thus explain the different lipophilicity and "bulkiness" requirements of verapamil on the one hand and these compounds on the other. Further investigation into the mechanism of action would aid in establishing the validity of this argument.

From the experiments, a general structure activity relationship emerged. Compounds with aromatic side-chains, with the exception of the imino-ketone **3b** (which had marginal activity), were all active. Aliphatic compounds with methyl or butyl side-chains (compounds **3a**, **4b** and **4c**) were inactive and also had "low" lipophilicities and less molecular surface area. However, aliphatic compounds with octyl side chains, such as compounds **3c** and **4d** were as active, or even more active, than their aromatic counterparts. From these results it would appear that bulky substituents are essential for calcium antagonistic activity. Furthermore, it became clear that binding with a calcium channel binding site, as measured by washout time, was greatly enhanced in compounds with aromatic substituents, a threefold increase in washout time was required for some aromatic compounds compared with their aliphatic counterparts (see Fig. 2). This finding may lead to the rational design of similar calcium antagonists where the duration of action may be manipulated by chemical modification, such as the inclusion or deletion of aromatic substituents on certain positions on the cage skeleton.

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