Quantitative Structure Activity Relationships of Catechol Derivatives on Nerve Growth Factor Secretion in L-M Cells

Angeliki Kourounakis[1] and Nicholas Bodor[1,2]

Received January 5, 1995; accepted February 24, 1995

Purpose. Although many catechol derivatives are potent stimulators of *Nerve Growth Factor* synthesis in L-M cells, not much is known about their mechanism of action. In order to obtain a Quantitative Structure Activity Relationship (QSAR), AM1 quantum mechanical calculations were performed on a group of 23 catechol derivatives with different levels of activity. ***Methods.*** A set of 18 parameters/descriptors were obtained by AM1 quantum mechanical calculations for each catechol derivative. Linear combinations of the calculated descriptors were fitted to the activity (as extracted from literature data) of the compounds by using simple or multiple regression analysis. ***Results.*** Good correlation with activity was obtained for specific parameters such as the adiabatic ionization potential and other 'oxidation'-related descriptors of the molecules while poor correlation was observed for most of the other parameters as, for example, for log P. ***Conclusions.*** Our results show that activity is associated with parameters related to the oxidation of the catechol derivatives, strongly supporting recent literature suggesting that an oxidative process is involved in their action.

KEY WORDS: catechol; L-M cells; NGF; AM1; QSAR; activity.

INTRODUCTION

The potential treatment of neurodegenerative diseases with Nerve Growth Factor (NGF)—inducing compounds has led to extensive demonstration of the inducibility of NGF in a number of *in vivo* and *in vitro* systems by a variety of compounds. Among them, it has been demonstrated that catecholamines stimulate NGF synthesis in the mouse fibroblast L-M cell line (1,2,3). Evidence indicates that their effect is due to the catechol part of their molecule and not mediated by adrenergic receptors, that are present in this cell line, since α or β adrenergic antagonists do not block their effect (1,2). In addition, m- or p- dehydroxy precursors of catecholamines (4- or 3- aminoalkyl phenols), m-O-methylated metabolites (2-methoxy-4-aminoalkyl phenols), or non-catechol adrenergic agonists showed no stimulatory effect on NGF content (1). On the other hand, 4-alkyl catechols (1, 2 dihydroxy-4-alkyl benzenes) such as 4-methyl catechol were shown to be very potent stimulators (4). The mechanism by which catechol or catecholamine analogs stimulate NGF synthesis has not been elucidated yet (5).

Some preliminary structure activity relationships of the catechol analogs suggested that the catechol ring is essential for the stimulatory effect on NGF synthesis. From the structure-effect of the aliphatic side chain it was deduced that β-hydroxylation decreased, N-substitution (non-bulky) enhanced, while α-carboxylation decreased the stimulation effect on NGF synthesis (2). Also, shortening of the chain length progressively reduced activity (2) except in the case of alkyl side chains without any substituents, where the opposite was observed (4). The question, therefore, that arises is why the catechol moiety is responsible for activity and, furthermore, what specific characteristics of that moiety, as modified by varying substitution on the ring, influence that activity.

In order to study the effects of the side chain on the catechol moiety of the molecules and to correlate characteristics of the molecular structure with activity, a set of 23 substituted catechols, with activities indicated in literature data, were characterized by various electronic, steric, and thermodynamic factors derived from the semi-empirical AM1 method (6).

MATERIALS AND METHODS

The entire set of 23 compounds was calculated by the Tektronix CAChe (Computer Assisted Chemistry) workstation. Conformational analysis was performed by AM1 quantum mechanics. In order to reach the low energy conformation of the catechol moiety of the 4-substituted catechols, 4-methyl-catechol and isoproterenol were used as models: the relative position of the two H atoms of the two phenolic OHs was determined by interactively rotating the two O-H bonds. The dihedral angles, defined by the ring plane and the HO-C plane, were estimated respectively. As shown in figure 1, the conformations with the lowest energy had both H atoms on the plane of the benzene ring (dihedral angles 0 or 180 degrees). In addition, the orientations of the two Hs were such that two low energy conformations were obtained (for the 4-substituted catechols) by the two possible arrangements of the apparent intramolecular H-bond between the two phenolic OH groups: the conformer 1A, with O meta as proton donor, and the conformer 1B, with O para as the proton donor (figure 1).

1A was chosen based on the premise that catechols interact with adrenergic receptors in a similar form (7), and it was used as the low energy conformation in all subsequent calculations.

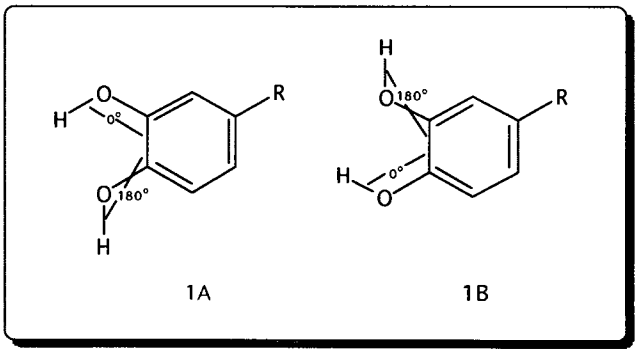
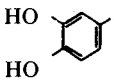
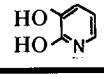


Fig. 1. The two low energy conformations of 4-alkyl catechols.

[1] Center for Drug Discovery, College of Pharmacy, P.O. Box 100497, University of Florida, Gainesville, Florida 32610-0497.
[2] To whom correspondence should be addressed.

0724-8741/95/0800-1199$07.50/0

Table I. The Set of 23 Catechol Derivatives and Values of Their Descriptors

	Compound	Structure	CpO[a]	CmO[b]	DM[c] (debye)	log P[d]	V[e] (Å^3)	S[f] (Å^2)	O[g]	IPa[h] (kcal)	IPv[i] (kcal)
1	4-MC	 R = $-CH_3$	0.271	0.249	2.074	1.351	116.297	151.134	1.312	190.016	202.055102
2	3-MC		0.273	0.249	2.41	1.336	116.065	149.386	1.298	191.38	204.384201
3	4-EC	R = $-CH_2-CH_3$	0.271	0.25	2.109	1.766	132.95	171.399	1.361	189.672	201.847559
4	4-PC	R = 	0.272	0.249	2.11	2.192	149.755	192.885	1.414	189.422	201.847559
5	4-BC	R = 	0.271	0.249	2.115	2.624	166.617	214.692	1.466	189.347	201.824498
6	4-tBC	R = $-C(CH_3)_3$	0.271	0.25	2.071	2.498	166.021	207.795	1.422	188.982	202.078163
7	DBA	R = $-COOH$	0.264	0.241	4.154	0.925	126.523	162.332	1.332	200.896	214.48465
8	DHB	R = $-CHO$	0.266	0.246	2.802	0.991	118.79	153.006	1.309	199.431	212.755121
9	DHBA	R = 	0.272	0.25	0.975	0.465	128.192	166.525	1.354	189.219	203.231182
10	DHCA	R = 	0.266	0.245	4.031	1.689	154.314	197.189	1.417	197.134	208.788735
11	DHPPA	R = 	0.269	0.246	3.411	1.863	160.39	207.619	1.454	193.846	206.943904
12	DOPS	R =	0.268	0.245	3.896	0.37	180.161	227.329	1.474	191.922	208.834856
13	DOPAC	R = 	0.269	0.247	1.063	1.525	143.315	183.143	1.383	193.689	207.220629
14	DOPAMINE	R = 	0.271	0.249	1.413	0.894	144.959	188.955	1.416	191.0597	203.300363
15	DOPEG	R = 	0.27	0.246	3.626	1.507	149.392	191.539	1.407	191.372	204.130537
16	DOMA	R =	0.267	0.244	2.625	1.152	151.703	194.096	1.411	195.9671	212.017189
17	DOPA	R = 	0.268	0.247	3.968	0.693	172.02	220.772	1.476	190.3469	209.226882
18	EPINEPH	R = 	0.271	0.251	1.153	1.068	170.43	219.122	1.474	190.264	202.977518
19	ISO	R = 	0.271	0.246	2.775	1.673	204.377	261.95	1.561	190.7759	203.046699
20	NOREPIN	R = 	0.271	0.246	3.288	0.623	152.819	194.649	1.408	189.728	203.346484
21	EPININE	R = 	0.271	0.249	1.416	1.209	162.382	211.484	1.469	190.853	203.300363
22	PYROCAT	R = $-H$	0.271	0.248	2.128	0.933	99.632	129.864	1.25	193.101	204.89153
23	DHP		0.2669	0.2412	0.6577	−0.572	95.456	125.369	1.241	198.1156	209.987875

[a] The negative charge at the *para*-O.
[b] The negative charge at the *meta*-O.
[c] The dipole moment in debye units.
[d] The calculated log P (BLOGP program).
[e] The calculated molecular volume in Å^3.
[f] The calculated molecular surface in Å^2.
[g] The calculated ovality of the molecule.
[h] The adiabatic ionization potential in kcal units.
[i] The vertical ionization potential in kcal units.

Parameters/descriptors of the molecules, derived from the AM1 calculated data, were: the charge at the *para*- or *meta*- O (CpO, CmO), the dipole moment in debye (DM), the vertical ionization potential (IPv) in kcal units, the contribution of the *para*- or *meta*- O to the HOMO (highest occupied molecular orbital) (HpO, HmO), and the LUMO (lowest unoccupied molecular orbital)-HOMO difference in e.V. units (HARD). Based on the AM1 optimized geometry and the van der Waals radii of each atom, the molecular volume (V), surface (S), and ovality (O), as well as the lipophilicity, expressed as log P, were obtained using the BLOGP program (8,9). The ovality of the molecule (O), obtained from the calculated molecular volume and surface, is defined as the ratio of the actual surface, and the minimum surface:

$$O = S / [4\pi(3V/4\pi)^{2/3}]$$

where S is the molecular surface and V is the molecular volume.

In addition, other descriptors included: 1) the adiabatic ionization potential (IPa) in kcal units, defined as the difference between the heat of formation of the radical cation of the molecule and the heat of formation of the neutral molecule. The radical cation located on the *para*-OH of the molecule was chosen as the most stable after comparison with

Table I. Continued

	Compound	HpO[j]	HmO[k]	HARD[l] (e.V.)	Q[m] (kcal)	DMa[n] (debye)	IPva[o] (e.V.)	CpOa[p]	CmOa[q]	SAQ[r] (kcal)	Activity[s] (mM)
1	4-MC	0.3185	0.3255	9.0563	−42.801	4.12	2.758	0.5435	0.3061	232.817	0.026
2	3-MC	0.367	0.2963	9.1244	−43.265	3.965	2.763	0.5502	0.3054	234.645	0.057
3	4-EC	0.3182	0.3226	9.0596	−42.458	5.708	2.789	0.5423	0.306	232.13	0.055
4	4-PC	0.3181	0.3232	9.0585	−42.449	7.729	2.8136	0.5408	0.306	231.871	0.05
5	4-BC	0.3183	0.3218	9.0589	−42.4233	9.779	2.824	0.5405	0.3056	231.7703	0.096
6	4-tBC	0.3155	0.3347	9.0984	−42.5808	8.009	2.84	0.5407	0.3057	231.5628	0.129
7	DBA	0.2953	0.389	9.9007	−46.278	3.377	3.616	0.4914	0.2946	247.174	1.307
8	DHB	0.2994	0.3744	8.6578	−46.3551	1.9555	3.562	0.495	0.2991	245.7861	1.33
9	DHBA	0.3097	0.3448	9.0525	−43.5929	5.383	2.7148	0.3201	0.3407	232.8119	0.531
10	DHCA	0.3072	0.2945	8.96799	−45.09288	4.01599	3.69199	0.474	0.2928	242.226	0.314
11	DHPPA	0.3156	0.3456	9.04258	−43.7481	9.5547	3.0782	0.5302	0.3028	237.594	0.121
12	DOPS	0.307	0.3628	9.03835	−43.0147	10.7069	3.2725	0.5192	0.2998	234.9367	0.122
13	DOPAC	0.3117	0.35068	9.18863	−45.179	7.0784	3.1526	0.5232	0.3017	238.868	0.572
14	DOPAMINE	0.3152	0.3351	9.0557	−43.453	7.0397	2.9024	0.5374	0.3058	234.5127	0.087
15	DOPEG	0.31203	0.35045	9.06639	−43.5755	8.60485	2.9914	0.5319	0.3029	234.9475	0.161
16	DOMA	0.3097	0.3694	8.9861	−49.7407	6.5288	3.386	0.5136	0.2997	245.7078	0.825
17	DOPA	0.3148	0.336	8.97999	−44.381	8.91158	3.2455	0.5234	0.03012	234.7279	0.159
18	EPINEPH	0.3083	0.3385	9.02145	−44.7795	10.4316	2.9618	0.5336	0.3055	235.0435	0.092
19	ISO	0.311	0.3471	9.0745	−43.1239	13.555	2.9837	0.5321	0.303	233.8998	0.148
20	NOR	0.3111	0.3465	9.0957	−42.0931	8.3238	2.9735	0.5332	0.3025	231.8211	0.151
21	EPININE	0.3152	0.3362	9.05548	−43.4935	9.09112	2.9068	0.5371	0.3052	234.3465	0.075
22	PYROCAT	0.366	0.3085	9.18215	−43.6833	2.6293	2.7459	0.5486	0.3072	236.7843	0.984
23	DHP	0.3692	0.31015	9.1339	−46.8859	3.6432	3.1327	0.515	0.299	245.0015	1.33

[j] The contribution of the *para*-O to the HOMO (absolute values).
[k] The contribution of the *meta*-O to the HOMO (absolute values).
[l] The LUMO-HOMO difference in e.V. units.
[m] The difference between the heat of formation of the catechols and the corresponding quinones in kcal units.
[n] The dipole moment of the anion in debye units.
[o] The vertical ionization potential of the anion in e.V. units.
[p] The negative charge at the *para*-O of the anion.
[q] The negative charge at the *meta*-O of the anion.
[r] The sum of the absolute values of IPa and parameter Q.
[s] The activity expressed as concentration (mM units).

the corresponding *meta*-, 2) the parameter "Q" (in kcal units) defined as the difference between the heat of formation of the catechols and the corresponding quinones, and, 3) the parameter "SAQ" (in kcal units) generated from the sum of the absolute values of IPa and parameter "Q".

Finally, parameters from the catechol anion, (negative charge at the *para*-O), that were used were the dipole moment (DMa) in debye units, the vertical ionization potential (IPva) in e.V. units, and the charge at the *para*- or *meta*-O (CpOa, CmOa).

Activity was expressed as concentration (mM) of the compound needed in order to increase 10 fold the NGF content in the media of the L-M cell culture after 24h incubation. Based on literature data (references 1, 2, and 4), this concentration was deduced after regression analysis of the initial linear part of the bell shaped curve of activity (fold increase

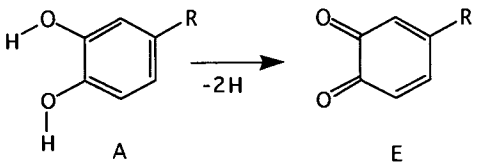


Fig. 2. Oxidation of a catechol derivative to the quinone.

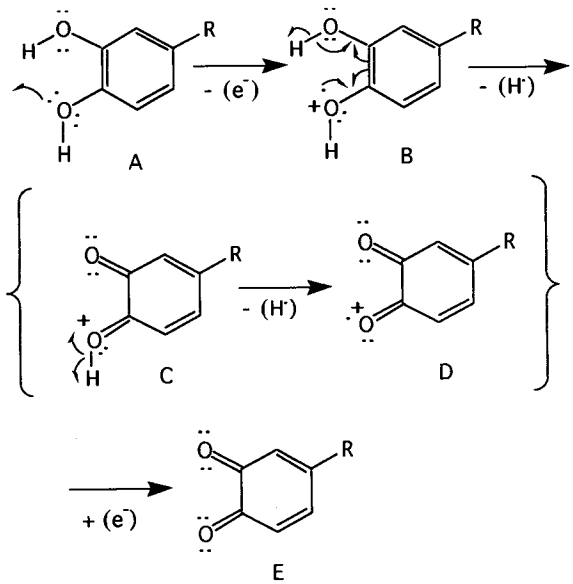


Fig. 3. A probable mechanism of oxidation of a catechol derivative to the corresponding quinone.

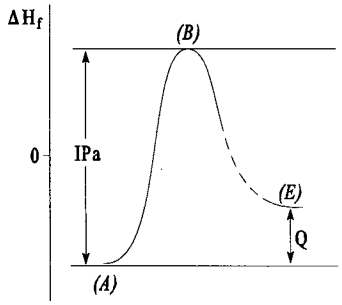


Fig. 4. Hypothetical energetic diagram of the catechol oxidation.

of NGF content) vs. concentration of compound. In the presence of 0 to 0.2 mM of each compound, the increase of NGF content in the cell culture medium ranged from 1 to 16.5 fold depending on the compound. All values, extracted from the literature data, were the mean of four determinations ± SE (with SE ranging from 0.07 to 3) as indicated therein. Linear regression of the fold increase and the concentration of each compound gave excellent correlation (R = 0.92 to 0.99) and from the derived equations the concentration of each compound required for 10 fold NGF-increase was determined. For example, the activity of 4-methylcatechol (4-MC) (4) and epinephrine (EPINEPH) (2) were calculated as shown below:

4-MC:

Fold increase in NGF ± S.E. (x)	Concentration (mM) (y)
1 (±0)	0
7 (±0.5)	0.02
12.5 (±2.5)	0.04

Regression analysis gave $y = 0.003\,x - 0.004$ ($R = 1$). Thus for 10 fold increase in NGF, $x = 10$, y (the concentration in mM) is 0.026.

Table II. The Correlation Matrix

	CpO	CmO	DM	log P	V	S	O	IPa	IPv
CpO	1								
CmO	0.82308054	1							
DM	−0.47828417	−0.45151477	1						
log P	0.32167374	0.45550544	0.10324916	1					
V	0.10315528	0.14715862	0.30927386	0.38661558	1				
S	0.11493417	0.15608032	0.29085196	0.38122848	0.99856073	1			
O	0.1373125	0.18977752	0.25723836	0.38488327	0.98632353	0.99297205	1		
IPa	−0.88139881	−0.80271569	0.26799565	−0.35156284	−0.39700784	−0.40243585	−0.41880759	1	
IPv	−0.92839064	−0.81138702	0.42897824	−0.44860345	−0.2082015	−0.21969371	−0.24447794	0.89336367	1
HpO	0.30362141	0.01068673	−0.36418956	−0.25548451	−0.56656427	−0.56133185	−0.57864382	−0.02079504	−0.14550899
HmO	−0.46136385	−0.36979923	0.29024817	−0.15087781	0.24293972	0.23368817	0.22539149	0.32217372	0.49074782
HARD	−0.23032845	−0.37615973	0.14867017	−0.0735475	−0.17106706	−0.1769704	−0.18827304	0.26671823	0.20609073
Q	0.72334518	0.61734051	−0.01303477	0.38377965	0.24996831	0.25132984	0.25496573	−0.77306685	−0.80679466
DMa	0.32084544	0.21113017	0.08887351	0.28421185	0.90954703	0.91643557	0.9145203	−0.5542862	−0.39019812
IPva	−0.94018895	−0.73502334	0.56726236	−0.22194061	0.07655588	0.06303964	0.03965546	0.8179199	0.88326788
CpOa	0.1551935	0.0595794	0.09833698	0.32152063	0.14436251	0.14192812	0.12480961	−0.13879249	−0.19258064
CmOa	0.23487097	0.09184277	−0.3795467	0.15425195	−0.22706184	−0.22882941	−0.22686821	0.04917732	−0.26233762
SAQ	−0.87322686	−0.78036231	0.1910469	−0.38258746	−0.3660305	−0.37029503	−0.38298814	0.97359316	0.91157978
activity	−0.67487574	−0.65009661	−0.04059223	−0.49120002	−0.58872098	−0.59454285	−0.61882941	0.83464166	0.74984145

Table II. Continued

	HpO	HmO	HARD	Q	DMa	IPva	CpOa	CmOa	SAQ	activity
CpO										
CmO										
DM										
log P										
V										
S										
O										
IPa										
IPv										
HpO	1									
HmO	−0.6829623	1								
HARD	0.0222324	0.2198868	1							
Q	0.0437993	−0.3657257	−0.0616437	1						
DMa	−0.3523976	0.1791437	−0.1384666	0.3658255	1					
VIPa	−0.4217543	0.4442453	0.09659	−0.6746447	−0.2107311	1				
CpOa	0.2598702	−0.1986461	−0.0045589	0.1973119	0.2312841	−0.1512141	1			
CmOa	0.0506113	0.0019207	0.0903044	0.0801506	−0.1373285	−0.2384518	−0.1241779	1		
SAQ	−0.0302232	0.3556525	0.2076537	−0.8974626	−0.517094	0.8115586	−0.1675239	0.0053506	1	
activity	0.1561201	0.3311464	0.265348	−0.7248103	−0.638539	0.5213607	−0.2909935	0.079332	0.8412413	1

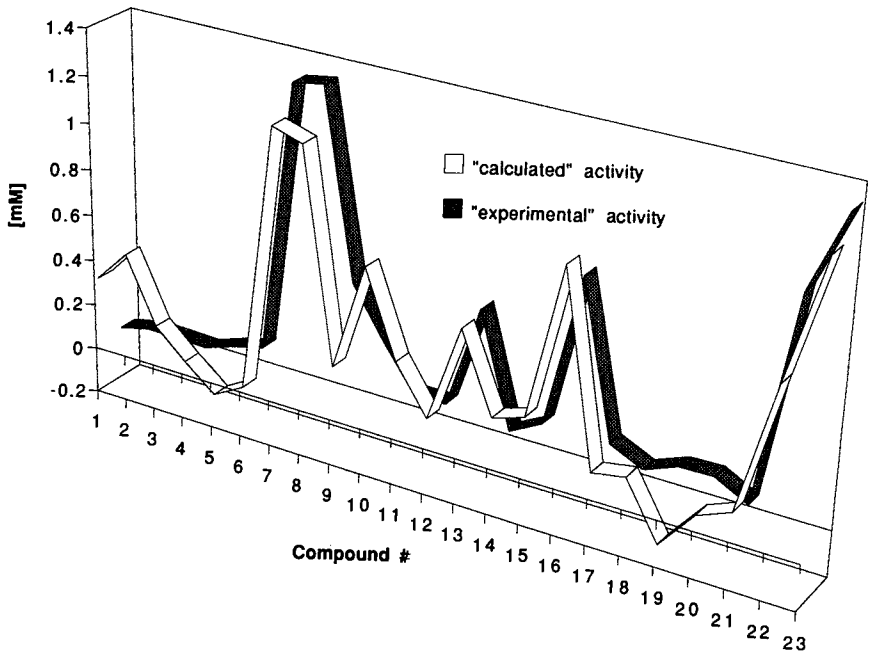


Fig. 5. "Calculated" activity values fitted close to the "experimental" values. Compound #s correspond to those in Table I.

EPINEPH:

Fold increase in NGF ± S.E. (x)	Concentration (mM) (y)
1 (±0)	0
6.7 (±0.5)	0.05
11 (±0.6)	0.1
14 (±2)	0.15

Regression analysis gave y = 0.011 x − 0.018 (R = 0.99). Thus for 10 fold increase in NGF, x = 10, y (the concentration in mM) is 0.092.

Linear combinations of the calculated descriptors were fitted to the calculated activity by using simple or multiple regression analysis.

RESULTS AND DISCUSSION

The set of 23 catechol derivatives (with abbreviations) included: 4-Methyl-Catechol (4-MC), 3-Methyl-Catechol (3-MC), 4-Ethyl-Catechol (4-EC), 4-Propyl-Catechol (4-PC), 4-Butyl-Catechol (4-BC), 4-tButyl-Catechol (4-tBC), 3,4-Dihydroxybenzoic acid (DBA), 3,4-Dihydroxybenzaldehyde (DHB), 3,4-Dihydroxybenzylamine (DHBA), 3,4-Dihydroxycinnamic acid (DHCA), 3,4-Dihydroxyphenylpropionic acid (DHPPA), 3,4-Dihydroxyphenylserine (DOPS), 3,4-Dihydroxyphenylacetic acid (DOPAC), Dopamine, 3,4-Dihydroxyphenylglycol (DOPEG), 3,4-Dihydroxymandelic acid (DOMA), 3,4-Dihydroxyphenylalanine (DOPA), Epinephrine (EPINEPH), Isoproterenol (ISO), Norepinephrine (NOREPIN), Epinine, Pyrocatechol (PYROCAT), and 2,3-Dihydroxypyridine (DHP).

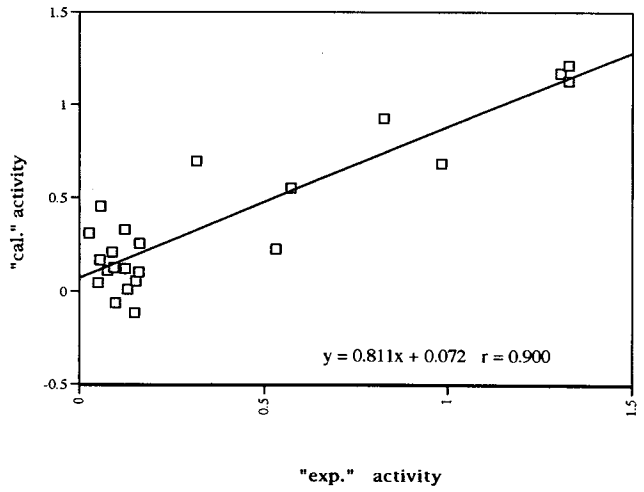


Fig. 6. "Experimental" values of activity ("exp." activity) plotted against "calculated" values ("cal." activity).

Structures of the compounds and values of the parameters calculated for each compound are shown in Table I.

The correlation matrix is represented in Table II, where absolute values are the correlation coefficients (multiple R).

Initially, the parameter that gave the best simple correlation with activity was the adiabatic ionization potential (IPa) (equation 1), followed by the vertical ionization potential (IPv) (equation 2) and the Q (equation 3).

$$\text{Activity [mM]} = -20.2391 + 0.10713\ \text{IPa} \quad (1)$$
$$n = 23,\ R = 0.8346,\ \text{s.e.} = 0.2543,\ F = 48.2216$$
$$\text{Activity [mM]} = -17.4749 - 0.0867\ \text{IPv} \quad (2)$$
$$n = 23,\ R = 0.7498,\ \text{s.e.} = 0.3055,\ F = 26.9739$$
$$\text{Activity [mM]} = -7.5492 - 0.1798\ Q \quad (3)$$
$$n = 23,\ R = 0.7248,\ \text{s.e.} = 0.3181,\ F = 23.2431$$

where n is the number of compounds submitted to the regression, R is the correlation coefficient, s.e. is the standard error, and F is the overall statistical significance of the equation.

Poor correlation was observed with all other parameters, for example with log P (equation 4) or HARD (e.V.) (equation 5).

$$\text{Activity [mM]} = 0.7649 - 0.3083\ \text{lop P} \quad (4)$$
$$n = 23,\ R = 0.4912,\ \text{s.e.} = 0.4022,\ F = 6.6781$$

$$\text{Activity [mM]} = -4.9364 + 0.5851\ \text{HARD} \quad (5)$$
$$n = 23,\ R = 0.2653,\ \text{s.e.} = 0.4452,\ F = 1.5906$$

where HARD, twice the absolute hardness of the molecule (as expressed by the calculated energy difference between the LUMO and HOMO), is an electronic descriptor of the polarizability of molecules (10).

The values of the adiabatic ionization potential (IPa) and of the parameter Q can be considered to describe respectively a transition state and the final product of the oxidation of a catechol (A) to the corresponding quinone (E), (Figure 2). A probable mechanism describing this oxidation is shown in Figure 3.

From the IPa and Q values (Table I) we can deduce that the energy (expressed as heat of formation) of the radical cation of the catechol (B) is the highest followed by the energy of the quinone (E) and then by the catechol (A). The hypothetical energetic diagram for the oxidation of the catechol via the transition state to the ortho quinone is depicted in the diagram of Figure 4.

These observations/hypotheses led us to relate the sum of the absolute values of IPa and parameter Q, (expressed as SAQ), with activity, which gave the best simple correlation compared to all other parameters (Table II).

The correlation of activity with the descriptor SAQ (e.V.) is shown in equation 6:

$$\text{Activity [mM]} = -17.3832 + 0.07508\ \text{SAQ} \quad (6)$$
$$n = 23,\ R = 0.8412,\ \text{s.e.} = 0.2496,\ F = 50.8407$$

The best relationship between activity and descriptors employs, in addition to SAQ, a geometrical descriptor of the catechol derivatives and is given by equation 7:

$$\text{Activity [mM]} = -11.8130 + 0.0632\ \text{SAQ} - 1.9763\ \text{O} \quad (7)$$
$$n = 23,\ R = 0.9004,\ \text{s.e.} = 0.2058,\ F = 42.8571$$

where O is the ovality of the molecule that is dimensionless.

Both the 'calculated' and 'experimental' values of activity from equation (7) are shown in Figure 5, where a good fit is observed.

Figure 6 shows the 'experimental' activity values plotted against our calculated values.

CONCLUSION

The attempt to correlate activity of catechol derivatives with few molecular or electronic descriptors was successful: good correlation was found between NGF stimulatory activity in L-M cells and the IPa or SAQ descriptors. This strongly suggests that activity is related to the oxidative capacity of the catechol moiety since NGF stimulation increases along with the increase in the oxidative potential of the catechol (equations 1, 6, and 7). The easier the catechol can be oxidized, the more active it is. Thus, using descriptors of oxidation, such as IPa or SAQ, an estimate of activity or inactivity of novel catechol derivatives may be obtained.

Furthermore, since the mechanism responsible for the stimulation of NGF by catechol derivatives is still under investigation, our results support recent findings that suggest an oxidation process involved in this stimulatory effect (11). Since it was reported that antioxidants prevented the stimulatory effect of the catechol derivative DOPS, while several quinone derivatives increased NGF content (11,12), it was strongly suggested that the stimulatory effect of catechol derivatives on NGF secretion in L-M cells is predominantly mediated by the quinones formed by autoxidation in the culture of these cells. Thus, NGF may be induced by oxidative stress as a protective response of cells.

These findings are of particular interest when taking under consideration the fact that catechol derivatives also stimulate NGF in vivo (13), (in rat brain) (14), and that quinones are most probably formed also in vivo. Thus, not only the mechanism of NGF stimulation but also the protective role of NGF itself on neurons becomes an intriguing question to resolve, especially when considering that the cause of degeneration of NGF-responsive neurons in diseases such as Alzheimer's is yet to be determined.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Ming-Ju Huang for her valuable technical assistance, Dr. V. J. Demopoulos and Dr. J. Simpkins for helpful discussions. This work was partially supported by the NIH Grant PO1-AG10485.

REFERENCES

1. Y. Furukawa, S. Furukawa, and E. Satoyoushi. Catecholamines induce an increase in nerve growth factor content in the medium of mouse L-M cells. *J. Biol. Chem.* **261**:6039–6045 (1986).
2. Y. Furukawa, S. Furukawa, F. Ikeda, E. Satoyoushi, and K. Hayashi. Aliphatic side chain of catecholamine potentiates the stimulatory effect of the catechol part on the synthesis of nerve growth factor. *FEBS Lett* **208**:258–262 (1986).
3. Y. Furukawa, N. Tomioka, E. Satoyoushi, K. Hayashi, S. Furukawa. Catecholamines increase nerve growth factor mRNA content in both mouse astroglial cells and fibroblast cells. *FEBS Lett* **247**:463–467 (1989).
4. Y. Furukawa, N. Fukazawa, Y. Miyama, K. Hayashi, S. Furukawa. Stimulatory effect of 4-alkylcatechols and their diacetylated derivatives on the synthesis of nerve growth factor. *Biochem. Pharmacol.* **40**:2337–2342 (1990).
5. S. Carswell, E. K. Hoffman, K. Clopton-Hartpence, H. M. Wilcox, and M. E. Lewis. Induction of NGF by isoproterenol, 4-methylcatechol and serum occurs by three distinct mechanisms. *Mol. Brain Res.* **15**:145–150 (1992).
6. A. A. Larsen. Catecholamine Chemical Species at the Adrenergic Receptors. *Nature,* **224**:25–27 (1969).
7. M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, and J. J. P. Stewart. AM1: A New General Purpose Quantum Mechanical Molecular Model. *J. Am. Chem. Soc.,* **107**:3902–3909 (1985).
8. N. Bodor, Z. Gabanyi, and C.-K. Wong. A New Method for the Estimation of Partition Coefficient. *J. Am. Chem. Soc.* **111**: 3783–3786 (1989).
9. N. Bodor, M.-J. Huang. An Extended Version of a Novel Method for the Estimation of Partition Coefficients *J. Pharm. Sci.,* **81**:272–281 (1992).
10. R. G. Pearson. Absolute electronegativity and hardness correlated with molecular orbital theory. *Proc. Natl. Acad. Sci.* **83**:8440–8441 (1986)
11. A. Murakami, T. Tatsuno, T. Kimura, H. Noguchi, and M. Nakamura. Regulation of nerve growth factor secretion in L-M cells by catechol derivatives. *Neurosci. Res.* **17**:71–75 (1993).
12. R. Takeuchi, K. Murase, Y. Furukawa, S. Furukawa, and K. Hayashi. Stimulation of nerve growth factor synthesis/secretion by 1,4-benzoquinone and its derivatives in cultured mouse astroglial cells. FEBS **261**:63–66 (1990)
13. K. Kaechi, Y. Furukawa, R. Idegami, N. Nakamura, F. Omae, Y. Hashimoto, K. Hayashi, and S. Furukawa. Pharmacological Induction of Physiologically Active Nerve Growth Factor in Rat Peripheral Nervous System. *J. Pharmacol. Exp. Ther.* **264**:321–326 (1993).
14. M. S. Saporito, H. M. Wilcox, K. C. Hartpence, M. E. Lewis, J. L. Vaught, and S. Carswell. Pharmacological Induction of Nerve Growth Factor mRNA in Adult Rat Brain. *Exp. Neurol.* **123**:295–302 (1993).