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Vascular effects of diphenylmethoxypiperidine-derived dopamine uptake inhibitors

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ABSTRACT

Vascular effects of 4-aryl methoxypiperidinol compounds previously shown to share with cocaine the ability to inhibit the dopamine transporter are described. All the compounds tested inhibit KCl-induced and noradrenaline-dependent contractions in mesenteric arteries ex vivo. Thus, diphenylpyraline and its analogs may have a role as therapeutic options for the treatment of some of the cardiotoxic effects of cocaine intoxications.

As a powerful sympathomimetic agent, cocaine exerts its rewarding activity by blocking the dopamine transporter (DAT) with the subsequent increase in dopamine (DA) concentrations in the synapse. The search for cocaine antagonists has been the focus of researchers and several compounds with DAT inhibiting activity have been described.[1] For example, benzotropine (BZT) analogs possess a diphenylmethoxy moiety and a piperidyl ring; these compounds were previously studied as potential pharmacotherapies for cocaine addiction.[2] Similar to cocaine, the well-known histamine H1 receptor antagonist diphenylpyraline (DPP) increases brain dopamine, possesses psychostimulant properties in mice[3] and also shares the same BZT structural features. The fact that DPP functions as a potent dopamine reuptake inhibitor without producing significant rewarding effects suggests that DPP and its analogs merit further study as potential candidates for pharmacotherapies for cocaine addiction. These observations prompted the synthesis of a series of DPP analogs and it was shown that they share with DPP the ability to inhibit the DAT.[4] In these DPP analogs, symmetrical *para* substituents of the benzene ring were found to be important for high potency in binding to the DAT.[4] In addition, several piperidine derivatives have been shown to block an array of dopaminergic, serotoninergic and adrenergic monoamine transporters,[5] reinforcing the physiological relevance of the interactions between the piperidine structure and monoamine transporters.

Besides the well-established alterations in behavior, cocaine exerts powerful effects on the cardiovascular system with chest pain being one of the most common complaints with acute cocaine use.[6] Several studies have shown that cocaine increases contraction in isolated arteries and hearts,[7–9] supporting the notion that this increased contraction of coronary arteries caused by cocaine may be related to the myocardial infarction associated with acute cocaine intoxication.[10,11]

Since DPP and its analogs share with cocaine the inhibition of the DAT, we investigated whether these compounds share vascular properties with cocaine. In this study, the effects of equimolar doses of cocaine, DPP and its analogs were tested on KCl- and noradrenaline (NA)-induced contractions of the rat mesenteric resistance artery (MRA).

The DPP analogs were synthesized as previously described[4] utilizing methods used for the synthesis of the tropane series of compounds[12–14] (see Fig. 1). Final mixtures were purified by flash chromatography with mass and ^{1}H NMR spectra used for further analyses.[15]

Vascular contraction was tested in isolated mesenteric resistance arteries mounted in a wire myograph as previously described.[16] Arterial segments were normalized to 0.9· L_{100}, with L_{100} being the internal circumference the vessels would have if

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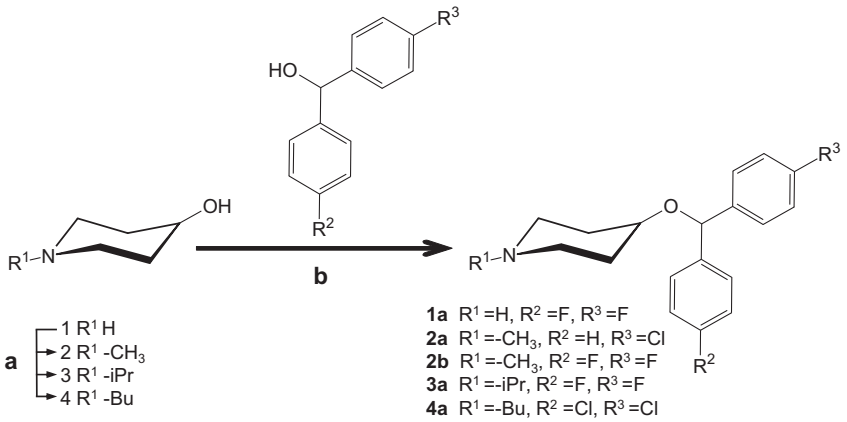

Figure 1. Synthesis of substituted diphenylmetoxypiperidines. Reagents and conditions: (a) Hal-R, K_2CO_3, DMF, 40 °C; (b) *p*-TSA, benzene/DMF (25:1), reflux. For details see Refs. 3,4.

they were exposed to a transmural pressure of 100 mmHg.[17] Optimal diameters (OD) were calculated as $OD = 0.9\ L_{100}/\pi$. Arteries with an OD of 278 ± 10 μm were used.[18]

The contraction to 75 mM KCl was used to evaluate receptor-independent responses, whereas receptor-dependent responses were evaluated as the contraction to a dose–response curve to NA. Maximal contraction to KCl was unaffected by pre-incubation with cocaine (3×10^{-6} M), however DPP (3×10^{-6} M) and all the diphenylmethoxypiperidines (each one at a concentration of 3×10^{-6} M) tested blocked KCl contraction (Fig. 2, $p < 0.05$). The diphenylmethoxypiperidines studied blocked KCl-induced contraction with varying efficacy. Compounds **3a** and **4a** were the most effective whereas **1a**, **2a** and **2b** share with DPP approximately a 70% of inhibition of KCl-induced contraction.

Dose response curves to noradrenaline (NA) in the presence of cocaine, DPP and diphenylmethoxypiperidine compounds are shown in Figure 3. Maximal responses to NA were not affected by pre-incubation with cocaine (Fig. 3A, Table 1), whereas **4a** was the only diphenylmethoxypiperidine compound that decreased NA maximal contraction (Fig. 3B, Table 1, $p < 0.05$). Sensitivity to NA was increased by cocaine and decreased by DPP and all diphenylmethoxypiperidines tested. The greatest inhibitory effect was observed with compounds **3a** and **4a** (Fig. 4, Table 1, $p < 0.05$). In the presence of cocaine the inhibitory effect of the diphenylmethoxypiperidine compounds on NA contraction remained unaffected (data not shown).

Parameters of the 'Rule of 5'[19] were calculated for the compounds tested using the online software Molinspiration.[20]

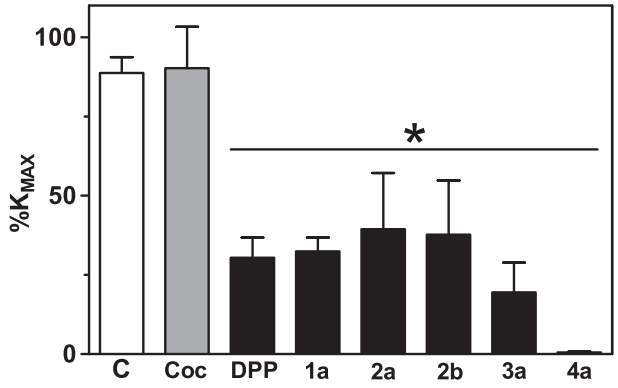

Figure 2. Effects of cocaine, DPP and diphenylmetoxypiperidines on KCl-induced contraction. MRA ($n = 6$) were exposed to 75 mM KCl during 5 min in the presence of cocaine (3×10^{-6} M), DPP (3×10^{-6} M) and DPP analogs (all at 3×10^{-6} M) as indicated. *$p < 0.05$ versus control arteries.

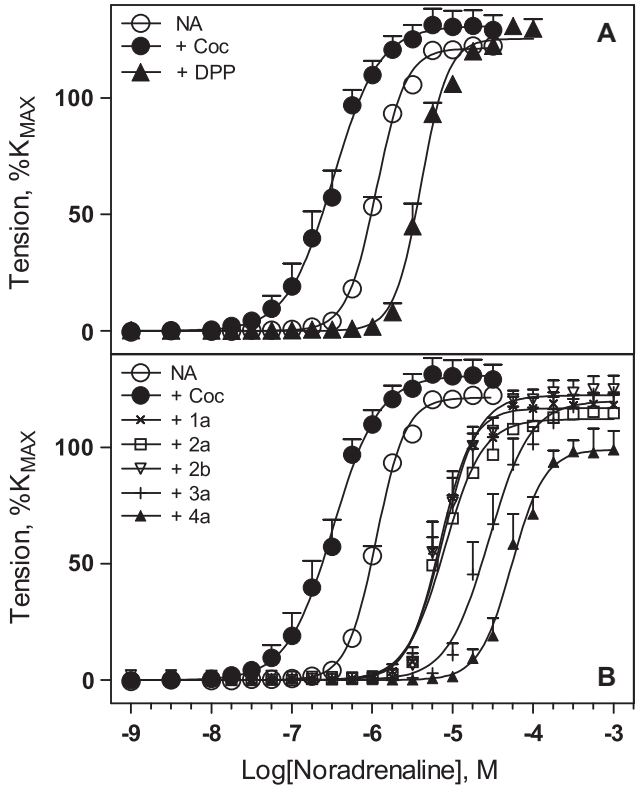

Figure 3. Effects of cocaine, DPP and diphenylmetoxypiperidines on NA-induced contraction. MRA were exposed to increasing concentrations of NA in the presence of cocaine (Coc, $n = 6$, 3×10^{-6} M) or DPP ($n = 6$, A, 3×10^{-6} M) and DPP analogs **1a**, **2a**, **2b**, **3a** and **4a**, ($n = 5$, B, all at 3×10^{-6} M) as indicated.

The relationship between the calculated octanol/water partition coefficient (Log*P*) and the observed effects on NA contraction were fitted to a linear regression. Compounds **3a** and **4a** with the greatest inhibitory effect on NA contraction display the largest Log*P* values (Table 1). Figure 5 shows the linear relationship between Log*P* and ΔpD_2 ($\Delta pD_2 = -0.3499 \cdot \text{Log}P + 0.3383$) suggesting that more effective inhibitors should possess increased lipophilicity.

Our results show for the first time that DPP and the DPP analogs tested inhibit receptor-dependent as well as receptor-independent contractions in MRA. Compared to cocaine, DPP and its analogs displayed opposite effects on vascular contraction; whereas cocaine treatment increases sensitivity to NA, all diphenylmethoxypiperidines compounds tested diminished sensitivity to NA.

Table 1
Effects of cocaine, DPP and DPP analogs on noradrenaline-mediated contractions in MRA

	R^1	R^2	R^3	Molecular Formula	pD_2	NA_{MAX} (% K_{MAX})	Log*P*
Noradrenaline					5.95 ± 0.03	122 ± 2	
Cocaine, 3×10^{-6} M					6.52 ± 0.09*	130 ± 7	
DPP	CH_3	H	F	$C_{19}H_{23}NO$	5.38 ± 0.04*	129 ± 4	3.161
1a	H	F	F	$C_{18}H_{19}F_2NO$	5.17 ± 0.07*	116 ± 4	2.869
2a	CH_3	H	Cl	$C_{19}H_{22}ClNO$	5.08 ± 0.08*	113 ± 8	3.815
2b	CH_3	F	F	$C_{19}H_{21}F_2NO$	5.10 ± 0.12*	121 ± 6	3.465
3a	*i*-Pr	F	F	$C_{21}H_{27}F_2NO$	4.48 ± 0.15*	121 ± 1	4.137
4a	Bu	Cl	Cl	$C_{22}H_{27}Cl_2NO$	4.26 ± 0.07*	99 ± 6*	5.931

Sensitivity to NA is expressed as pD2 and maximal contraction as % K_{MAX}. Calculated values of Log*P* are indicated for DPP and the DPP analogs studied.
* p <0.05 versus control arteries.

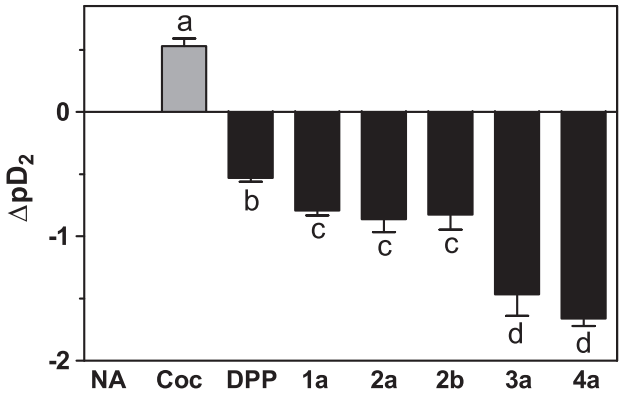

Figure 4. Effects of cocaine, DPP and diphenylmetoxypiperidines on sensitivity to NA-induced contraction. MRA were exposed to increasing concentrations of NA in the presence of cocaine (Coc, $n = 6$, 3×10^{-6} M), DPP ($n = 6$, 3×10^{-6} M) and DPP analogs ($n = 5$, all at 3×10^{-6} M) as indicated. Effects on NA sensitivity are expressed as ΔpD_2, letters indicate statistically significant differences.

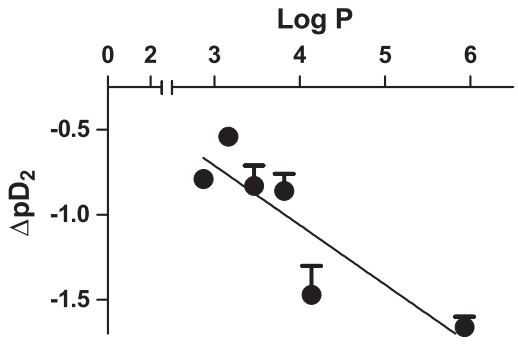

Figure 5. Correlation between lipophilicity and inhibition of NA contraction. Linear regression analysis between effects on NA sensitivity (ΔpD_2) and Log *P* for DPP and DPP analogs tested. $R^2 = 0.766$, p <0.02.

DPP and its analogs share with cocaine the ability to inhibit the DAT and our results point to a potential additional role of these compounds as therapeutic options for the treatment of the cardiotoxic effects of cocaine intoxications. The cocaine-induced increased sensitivity to NA in rat MRA has been previously described[21] and is consistent with its role as an inhibitor of catecholamines' reuptake by terminal nerves. This effect is ascribed to the blockade of the noradrenaline transporter and is potentially responsible for the increased coronary contraction as a contributor for the cardiotoxic effects observed in cocaine intoxications.[7,10,22,23] Moreover, cocaine in concentrations seen in drug users has also been shown to increase intracellular Ca^{+2} concentrations ($[Ca^{+2}]_i$) in cultured vascular smooth muscle cells from cerebral vessels.[24]

Our linear regression analysis showed that more active compounds should also be more lipophilic. The *N*-alkyl substituents appear to be more important than halogen substituents on the aromatic rings in terms of the activity described here. As the size of the *N*-alkyl substituent increases in compounds **1a** (–H), **2b** (–CH_3) and **3a** (–*i*Pr), all with *para*-F substituents in the aromatic rings, the inhibitory effect on NA contraction increases. The most effective compound **4a** has a *N*-butyl group, its activity may be due to the fact that the potential targets are integral membrane proteins such as ion channels and/or receptors, and agrees with the previously described importance of properly positioned lipophilic groups.[19]

Since DPP and its analogs are able to inhibit receptor-dependent and receptor-independent contractions, they may be acting upon global mechanisms of vascular control. The inhibitory effect is maintained in the presence of cocaine, suggesting that DPP and its analogs may act through different vascular mechanisms.

Vascular contraction, both receptor-dependent and receptor-independent, is mediated by rises in $[Ca^{+2}]_i$. As an inhibitor of the H1-histamine receptor, DPP and its analogs may interact with the complex vascular actions of histamine.[25,26] It was reported earlier that H1-antihistaminic compounds relax norepinephrine-contracted tissues;[27] the fact that this effect is also observed in KCl-contracted tissues[27] suggests that the inhibitory effects on adrenergic contraction are not mediated by direct interactions with α-adrenergic receptors. H1-antihistaminics have also been shown to modulate $[Ca^{+2}]_i$. The H1-antihistaminic astemizole lowers $[Ca^{+2}]_i$ by inhibiting Ca^{+2} influx in mast cells through the inhibition of store operated Ca^{+2} channels (SOC).[28] SOC channels are important in maintaining tonic contractions and are involved in adrenergic activation of the vasculature.[29] It is conceivable that DPP and its analogs modulate $[Ca^{+2}]_i$ by inhibiting SOC in MRA. However, the inhibitory effects of DPP and its analogs on NA contraction is not altered by cocaine suggesting an effect downstream direct modulation of $[Ca^{+2}]_i$.

Compounds with H1-antihistaminic activity have also been shown to block the K^+ channel human ether-a-go-go related gene HERG1 encoding the main subunit of a cardiac K^+ channel.[30] HERG1 activity is responsible for the rapid component of the ventricular repolarizing current I_{Kr} and the interaction of H1-antihistaminics with HERG1 has been suggested as responsible for the cardiotoxic effects displayed by some of these drugs.[31] Since HERG channels are expressed in smooth muscle cells[32], our results also emphasize the importance of examining the effects of DPP and its analogs on HERG channels in future studies. Due to the high structural homology between K^+ channels, it is possible that H1-antihistaminic compounds may interact with additional members of this important family of ion channels and modulate their activities. Thus, the effects of DPP and its analogs on Ca^{+2} and K^+ channels may be operating in the vasculature to inhibit KCl- and NA-dependent contractions.

We have shown that substituted diphenylmethoxypiperidines are able to inhibit both receptor-dependent and receptor-independent contractions in isolated MRA. The role of diphenylmethoxypiperidines on the regulation of $[Ca^{+2}]_i$ in vascular smooth muscle cells warrants further investigation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.04.040. These data include MOL files and InChiKeys of the most important compounds described in this article.

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14. N-substituted piperidin-4-ols **1–4** were prepared by alkylation with alkyl halides in *N*,*N*-dimethylformamide in the presence of anhydrous potassium carbonate in yields of approximately 90%. Commercially available substituted benzophenones were reduced to the benzhydrols using sodium borohydride in isopropanol in nearly quantitative yield. The ethers were synthesized by condensation of benzhydrols with 25% molar excess of N-substituted piperidin-4-ols or *N*-methylpiperidin-4-ol in benzene with a Dean–Stark trap. For details see Ref. 4.
15. For all compounds the mass spectra (Agilent 1100 HPLC and Micromass Quattro Ultima spectrometer; Waters, Milford, MA) contained weak $(M-H)^+$ ions characteristic of piperidines and the typical isotope patterns for one or more halogen atoms in the structures. Characteristic ions that occurred were *m*/*z* 99 (methylpiperidine fragment) as the base peak and *m*/*z* 114 (methylpiperidinol fragment) at approximately 50% R.A. in each mass spectrum. Significant diarylmethane fragments also occurred for all compounds. For compounds with very weak $(M-H)^+$ ions in the EI mass spectrum, electrospray ionization mass spectra from a methanol solution were taken to confirm the molecular weight as the $(M+H)^+$ ion. The relative response of all compounds was from 95.8% up to 99.1%. The ^1H NMR (300 MHz, $CDCl_3$, Varian EM-360 spectrometer) spectra of *N*-methylpiperidines showed typical signals: 1.30–1.35 (m, 2H, H-2,6 ax), 1.85–1.90 (m, 2H, H-2,6 equiv), 2.00–2.05 (m, 2H, H-3,5 ax), 2.40–2.45 (s, 3H, N-CH_3), 2.90–3.00 (m, 2H, H-3,5 equiv), 3.50–3.55 (m, 1H, H-4 ax), 5.35–5.45 (s, 1H, Ar-CH-Ar), 7.20–7.60 (m, aromatic C-H).
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18. For each arterial segment, the maximal response to KCl was calculated, and the response to NA was expressed as percent of the corresponding maximal response to KCl ($\%K_{MAX}$). Concentration-response curves to NA were analyzed by fitting individual experimental data to a logistic curve to determine maximal response and sensitivity. The curve was of the form $Y = \text{bottom} + (\text{top} - \text{bottom})/(1 + 10(\text{Log}\,EC_{50} - X) * \text{Hill Slope}))$ where *X* is the logarithm of the concentration and *Y* is the response; the sensitivity values reported are derived from these fits. Sensitivity was expressed as pD_2 ($pD_2 = -\log[EC_{50}]$) with EC_{50} being the concentration of agonist producing 50% of the maximal response. Effects on NA sensitivity were calculated for each experiment as the difference on sensitivity between NA and NA in the presence of the different compounds (ΔpD_2). One segment per animal was used to test each compound; *n* (between 5 and 6) refers to the number of animals used. Data are expressed as mean ± SEM. One-way analysis of variance (ANOVA) with Bonferroni's multiple comparisons was used to determine significant differences. A $p < 0.05$ was accepted as an indication of statistical significance. For details see Ref. 16.
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