British Journal of Pharmacology (2000) 129, 1561–1568

www.nature.com/bjp

Pharmacological comparison of the effect of ibogaine and 18-methoxycoronaridine on isolated smooth muscle from the rat and guinea-pig

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1 Ibogaine and 18-methoxycoronaridine are naturally occurring alkaloids reported to possess antiaddictive properties in several models of drug dependence. We have examined their effect at μ-opioid receptors regulating neurogenic contractions of several smooth muscle preparations and also against spontaneous contractions of the rat isolated portal vein.

2 Ibogaine (pIC_{50} 5.28) and 18-methoxycoronaridine (pIC_{50} 5.05) caused a concentration-dependent inhibition of cholinergic contractions of the guinea-pig ileum which was not affected by the opioid receptor antagonist naloxone (1 μM).

3 In the rat isolated vas deferens ibogaine and 18-methoxycoronaridine caused a concentration-dependent enhancement of purinergic contractions. Both agents (30 μM) caused a 3–5 fold rightward displacement of DAMGO-induced inhibition of purinergic contractions, but similar effects were observed for ibogaine against α_2-adrenoceptor-mediated inhibition of neurogenic responses.

4 In the guinea-pig isolated bladder both ibogaine (10 μM) and 18-methoxycoronaridine (10 μM) caused a 2 fold increase in the purinergic component of neurogenic contractions without significantly altering cholinergic contractions or responses to exogenous ATP. In contrast, ibogaine (1–30 μM), but not 18-methoxycoronaridine, caused a concentration-dependent enhancement of spontaneous contractions of the rat isolated portal vein.

5 In summary, while ibogaine and 18-methoxycoronaridine modulated electrically-evoked contractions in the three preparations examined, we have no evidence for a selective interaction with pre-junctional μ-opioid receptors. The pronounced enhancement of purinergic contractions produced by both agents is a novel finding and worthy of further investigation.

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Keywords: Ibogaine; 18-methoxycoronaridine; μ-opioid receptors; purinergic transmission; rat portal vein; guinea-pig isolated ileum; rat vas deferens

Abbreviations: DAMGO, [D-Ala^2, *N*-MePhe^4-Gly-ol] enkephalin; UK-14304, 5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline; K-H, Krebs-Henseleit

Introduction

Ibogaine is one of the psychoactive indole alkaloids found in a West African shrub, *Tabernanthe iboga*, which has been claimed to possess antiaddictive properties in man (see: Popik *et al*., 1995a). Studies in rats have demonstrated that self-administration of cocaine and morphine declined for several days after a single injection of ibogaine (Cappendijk & Dzoljic, 1993; Glick *et al*., 1991b; 1994; Maisonneuve *et al*., 1991). Also, ibogaine has been reported to reduce the withdrawal symptoms precipitated by administration of naltrexone in morphine-dependent rats (Glick *et al*., 1991a). The chief side effect of ibogaine appears to be a short-lived induction of tremors and ataxia, which is generally attributed to an interaction with sodium channels (Glick *et al*., 1994; Popik *et al*., 1995a). 18-Methoxycoronaridine is a congener of ibogaine which also possesses antiaddictive properties but is devoid of tremorigenic activity (Glick *et al*., 1996a).

While it is clear that the antiaddictive effect of ibogaine and 18-methoxycoronaridine is associated with an alteration in dopamine and 5-HT metabolism in the nucleus accumbens and striatum (Maisonneuve *et al*., 1991; Glick *et al*., 1994; 1996a; Benwell *et al*., 1996), the locus of action is not known. Ibogaine has been reported to interact with multiple sites (Deecher *et al*., 1992; Sweetman *et al*., 1995) with particular attention being paid to NMDA (Popik *et al*., 1995b), 5-HT (Sershen *et al*., 1997) and κ-opioid receptors (Glick *et al*., 1997). However, evidence is accumulating that μ-opioid receptors may be an important target for ibogaine.

Radioligand binding studies have revealed binding affinities for μ-opioid binding sites that vary from 130 nM to 26 μM (Deecher *et al*., 1992; Codd, 1995; Pearl *et al*., 1995; Sweetman *et al*., 1995). Also, Pablo & Mash (1998) reported that ibogaine behaves as a weak, partial agonist in a [^{35}S]-GTPγS binding assay for μ-opioid receptors. Further functional support for an interaction with μ-opioid receptors has been provided by the reports that ibogaine selectively enhances morphine-induced inhibition of adenylyl cyclase activity in rat striatum and hippocampus (Rabin & Winter, 1996) and morphine-induced antinociception in rats (Bagal *et al*., 1996). Surprisingly, however, the nature of the interaction with μ-opioid receptors has not been investigated in detail.

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In the present study we have examined the effect of ibogaine and 18-methoxycoronaridine, the non-tremorigenic congener, at μ-opioid receptors mediating inhibition of transmitter release in the rat vas deferens and the guinea-pig isolated ileum (Miller *et al*., 1986; Kosterlitz & Hughes, 1978). During the course of our investigation we noted that both alkaloids significantly enhanced purinergic contractions of the rat vas deferens and, therefore, decided to extend the study to another preparation with purinergic transmission, the guinea-pig isolated urinary bladder (Kasakov & Burnstock, 1983; Fujii, 1988), and also to the rat isolated portal vein.

Methods

The isolated tissues

Male Hooded Lister rats (200–300 g) were killed by carbon dioxide asphyxiation. The prostatic end of the vas deferens and a 2 cm segment of the portal vein were removed and placed in oxygenated, ice-cold modified Krebs-Henseleit (K-H) solution. The lower end of a 2 cm segment of the vas deferens or portal vein was secured to a plastic holder between parallel platinum wire electrodes, while the upper end was attached by cotton to a Grass FT-03 isometric transducer connected to a Grass Polygraph or a MacLab recording system (see below). The holder was placed in an isolated organ bath containing 20 ml modified K-H solution gassed with 95% O_2, 5% CO_2 and maintained at 37°C. For experiments involving the vas deferens, 0.1 μM prazosin was included in the bathing medium to block α_1-adrenoceptors. After an equilibration period of 30 min, 1 g wt. tension was slowly applied and the preparations allowed to relax to a final resting tension of 0.3–0.4 g wt. over 60 min. During this time the portal vein developed spontaneous contractions. Maximal neurogenic contractions of the vas deferens were elicited by transmural stimulation *via* platinum wire electrodes (single pulse 0.5 ms duration, 0.1 Hz, 25 volts) with a Digitimer multisystem D330 stimulator (Digitimer Ltd., U.K.) until the responses were stable.

Male Dunkin-Hartley guinea-pigs (500–1000 g) were killed by cervical dislocation and the cerebral cortex and bladder removed. The intestines were exteriorized, the ileo-caecal junction located and 5 cm of the terminal ileum discarded. Approximately 30 cm of the terminal ileum was removed and the lumen flushed with modified K-H solution. Four 2 cm long segments of the ileum were secured on to a perspex holder supporting two parallel platinum wire electrodes and placed in a 20 ml isolated organ bath containing modified K-H solution maintained at 37°C and gassed with 95% O_2/5% CO_2. The upper end of the ileum was attached to a Grass FT-03C isometric transducer and the signal amplified on a WPI TBM4 amplifier and displayed on a Gould BS 272 flatbed recorder. After 40 min equilibration, the segments were placed under 2 g wt. resting tension and allowed to relax for a further 40 min. Maximal contractions of the tissue were elicited by transmural stimulation using single pulses (0.1 Hz, 0.3 ms, 200 mA) delivered by a Digitimer multisystem D330 stimulator. The urinary bladder was also taken, placed in ice-cold K-H solution and the contents gently evacuated. The mucosa was removed from the urinary bladder by the method of Ambache & Zar (1970) and strips (1.5 cm long, 3 mm wide) prepared. The lower end of the strip was secured to a perspex holder between parallel platinum wire electrodes, while the upper end was attached by cotton to a Grass FT-03C isometric transducer. Changes in isometric tension were recorded by a MacLab 4e and displayed on a Macintosh LC475 computer. The holder was placed in an isolated organ bath containing 20 ml modified K-H solution gassed with 95% O_2/5% CO_2 and maintained at 37°C. After a 40 min equilibration period, each segment was placed under 3 g wt. tension and allowed to relax over the following 40 min. Preparations were stimulated with 60 mM KCl until reproducible responses were observed (each exposure separated by 30 min) and finally retensioned to achieve a final resting tension of 1.5 g wt. A Digitimer multisystem D330 stimulator was used to deliver 10 Hz, 2 s trains of electrical pulses (200 mA; 0.3 ms pulse width) every 5 min until the submaximal responses had stabilized (usually 90 min).

Cerebral cortices of guinea-pig brains were homogenized in 20 volumes of ice cold Tris buffer (50 mM Tris HCl; pH 7.4) in a Polytron PT disrupter (setting 6; 20–30 s). The homogenate was then centrifuged at 20,000 r.p.m. for 10 min at 4°C. The supernatant was discarded and the pellet resuspended in 20 volumes of 50 mM Tris buffer and recentrifuged. The supernatant was again discarded and the pellet resuspended in 4.9 volumes of 50 mM Tris buffer for direct use in binding assays or stored at −20°C.

The protocol

The effect of cumulative addition of either ibogaine or 18-methoxycoronaridine (1–30 μM) was examined against electrically-evoked contractions of the guinea-pig isolated ileum and rat vas deferens, and also against spontaneous contractions of the rat isolated portal vein. A minimum of 10 min was allowed between successive additions of the drugs. In the case of the ileum both agents were also examined in the presence of 1 μM naloxone. In the vas deferens, the effect of cumulative addition of DAMGO (10 nM–10 μM) against the electrically-evoked contractions was examined in the absence and presence of 30 μM ibogaine or 30 μM 18-methoxycoronaridine. In the guinea-pig isolated urinary bladder the effect of 10 μM ibogaine and 10 μM 18-methoxycoronaridine was examined against electrically-evoked contractions in the absence and presence of either 0.1 μM atropine or 300 μM suramin. In each case the receptor antagonists were added at least 30 min before the beginning of the experiment. Also, the effect of 10 μM ibogaine and 10 μM methoxycoronaridine was examined against contractions elicited by 500 μM ATP. In these experiments reproducible contractions to ATP were established using a protocol involving 5 min exposure followed by two washes of the tissue and 20 min re-equilibration. When the effect of ibogaine and 18-methoxycoronaridine was examined, these agents were added immediately following the washout of ATP.

The affinities of ibogaine, 18-methoxycoronaridine and atropine for muscarinic binding sites was determined by competition binding assay against 0.2 nM [^{3}H]-quinclidinyl benzilate (QNB) incubated with 100 μg protein. A range of ten concentrations of the competing ligand were examined in a total volume of 500 μl of Tris assay buffer (50 mM Tris HCl; pH 7.4 at 25°C). Non-specific binding was defined as percentage of bound ligand, compared to total binding, in the presence of 10 μM atropine (<5%). After an incubation period of 60 min at 25°C, bound radioactivity was separated from free by vacuum filtration over Whatman GF/B glass fibre filters using a Brandel cell harvester and quantified by liquid scintillation spectrometry.

Data analysis

Neurogenic contractions have been expressed as force (g wt.) or, when examining the effect of putative agonists, as a percentage of the pre-agonist responses. Contractions of the guinea-pig isolated bladder to 500 μM ATP have been expressed as a percentage of the contraction to 60 mM KCl. For the rat isolated portal vein the magnitude of spontaneous contractions (taken as the average over a 60 s period) in the absence of ibogaine or 18-methoxycoronaridine was compared to that observed prior to the addition of the drugs. All data has been given as the mean $\pm$ s.e.mean. Differences between mean values were considered statistically significant if $P<0.05$ for unpaired or paired observations (Student's *t*-test). The potency of the agonists in the absence and presence of the antagonists was assessed as the negative logarithm of the concentration required to cause 50% of the maximum response (pD_2) using the logistic equation described by DeLean *et al*. (1978) with Kaleidagraph software (Synergy) on a MacIntosh LC II computer. In addition, the inhibitory effect of ibogaine, 18-methoxycoronaridine and atropine against electrically-evoked contractions of the guinea-pig ileum have been represented as pIC_{50} values, the negative logarithm of the concentration required to reduce the response to 50% of the original.

The inhibition of the radioligand by competing ligands was analysed to estimate the IC_{50} (concentration of competitor displacing 50% of specifically bound radioligand) with a non-linear least squares programme Graphpad (ISI). The IC_{50} value was converted to an affinity constant (K_i) using the expression derived by Cheng & Prusoff (1973):

$$K_i = IC_{50}/(1 + [L]/K_d]$$

in this expression [L] and K_d represent the radioligand concentration and dissociation constant, respectively.

Drugs

The following compounds were used: DAMGO ([D-Ala^2, N-MePhe^4-Gly-ol] enkephalin, Bachem); prazosin HCl (Pfizer); UK-14304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline bitartrate, Pfizer); ibogaine HCl (RBI), 18-methoxycoronaridine HCl (synthesized by Dr M. Kuehne, University of Vermont, Burlington, VT, U.S.A.), suramin HCl (Bayer, Wuppertal, Germany); naloxone HCl (Sigma); atropine sulphate (Sigma), ATP sodium (Sigma). Prazosin (1 mM) was dissolved in 0.1 M lactic acid and dilutions made in distilled water. Ibogaine was dissolved in ethanol at a concentration of 10 mM, while 18-methoxycoronaridine HCL was dissolved at a concentration of 10 mM in an aqueous solution of 8 mM sodium phosphate. All other drugs were dissolved in distilled water and added to the organ baths in a volume of 0.1 ml or less. The composition of the modified Krebs-Henseleit saline was (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 11.1.

Results

Guinea-pig isolated ileum and rat isolated vas deferens

Electrically-evoked contractions of the guinea-pig isolated ileum (2.41 $\pm$ 0.32 g wt., $n=6$) and rat isolated vas deferens (0.62 $\pm$ 0.08 g wt., $n=4$) were abolished by 0.1 μM atropine ($n=5$) and 300 μM suramin ($n=4$, respectively). Figure 1a shows that ibogaine and 18-methoxycoronaridine produced a concentration-dependent inhibition of electrically-evoked contractions of the guinea-pig isolated ileum. Ibogaine was slightly more potent than 18-methoxycoronaridine (pIC_{50} 5.28 $\pm$ 0.07

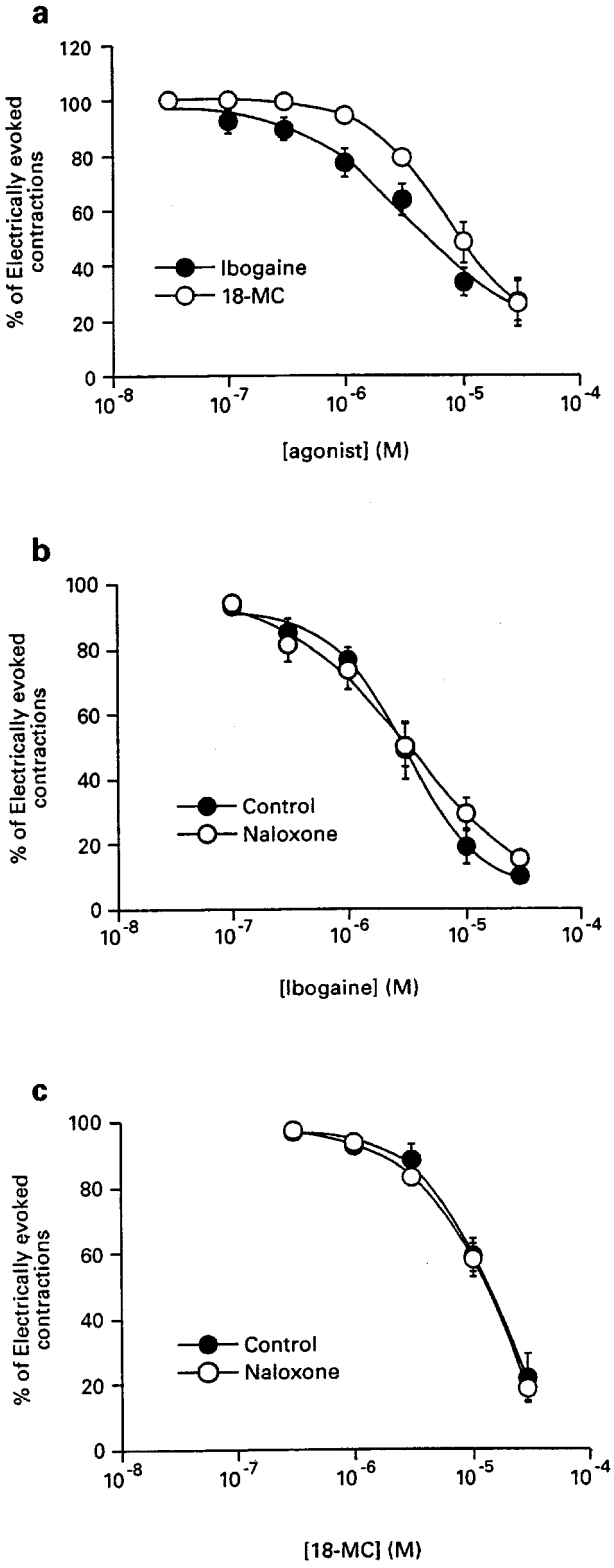

Figure 1 (a) A graph of the effect of ibogaine and 18-methoxycoronaridine (18-MC) against electrically-evoked (0.1 Hz) contraction of the guinea-pig isolated ileum. Responses have been expressed as a percentage of the control response and are shown as the mean $\pm$ s.e.mean of eight observations. (b,c) The effect of 1 μM nalaxone against (b) ibogaine-induced and (c) 18-methoxycoronaridine-induced (18-MC) inhibition of electrically-evoked (0.1 Hz) contractions of the guinea-pig isolated ileum. Responses have been expressed as a percentage of the control responses and are shown as the mean $\pm$ s.e.mean of six observations.

($n=8$) and 5.05 ± 0.11 ($n=8$), respectively) with 30 μM of each compound producing greater than 75% inhibition of the electrically-evoked contractions. Atropine (0.1 nM – 0.1 μM) also caused a concentration-dependent inhibition of the electrically-evoked contractions but was approximately 2000 fold more potent (pIC_{50} 8.61 ± 0.07, $n=5$) than ibogaine. At the highest concentration examined, the vehicle for ibogaine (0.3% v v^{-1} alcohol) did not significantly affect the electrically-evoked contractions.

Naloxone (1 μM), the opioid receptor antagonist, failed to modify the inhibitory effect of ibogaine (control pIC_{50} 5.53 ± 0.12; naloxone pIC_{50} 5.46 ± 0.08, $n=6$), and 18-methoxycoronaridine (control pIC_{50} 4.87 ± 0.10; naloxone pIC_{50} 4.97 ± 0.08, $n=6$), indicating no role for μ-opioid receptors in this effect (Figure 1b,c). Similarly, radioligand binding experiments with guinea-pig cerebral cortex membranes labelled with 0.2 nM [^{3}H]-QNB showed that 18-methoxycoronaridine had no affinity for muscarinic receptors ($pK_i<4$, $n=4$) while ibogaine exhibited low affinity (pK_i 4.63 ± 0.16, $n=5$). In contrast, atropine (pK_i 8.73 ± 0.10, $n=5$) was 10,000 fold more potent than ibogaine and 18-methoxycoronaridine.

Figure 2a shows that ibogaine and 18-methoxycoronaridine caused a concentration-dependent enhancement of electrically-evoked contractions of the rat vas deferens, with the maximum concentration employed (30 μM) increasing responses to $203\pm23\%$ ($n=5$) and $269\pm33\%$ ($n=6$), respectively, of control. The enhancement of neurogenic contractions was rapid, taking 4 – 5 min to reach equilibrium (Figure 2b).

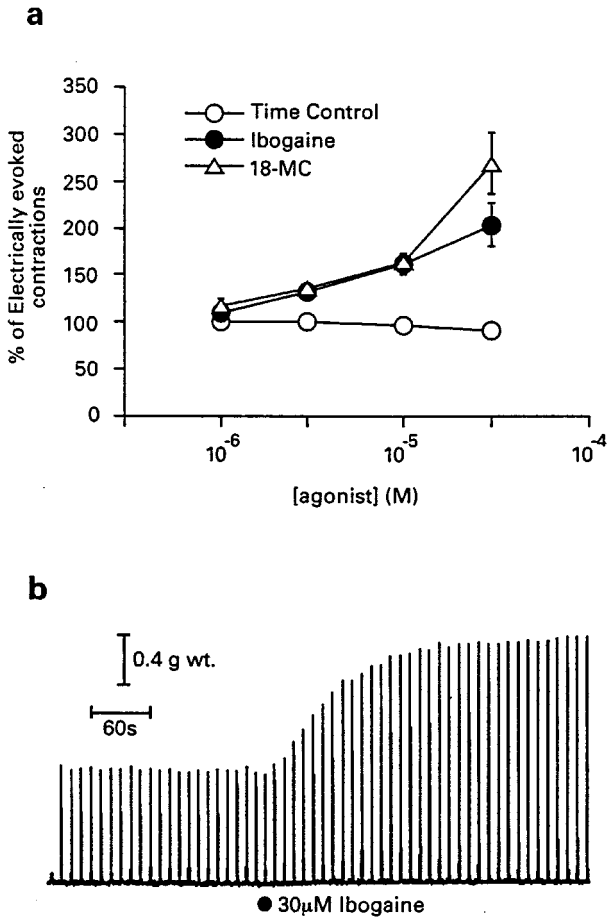

Figure 2 (a) A graph of the effect of ibogaine and 18-methoxycoronaridine (18-MC) on electrically-evoked (0.1 Hz) contractions of the rat isolated vas deferens. Responses have been expressed as a percentage of the control response and are shown as the mean $\pm$ s.e.mean of 5 – 6 observations. (b) Representative digitized recording of the effect of 30 μM ibogaine on electrically-evoked (0.1 Hz) contractions of the rat isolated vas deferens.

DAMGO, a selective μ-opioid receptor agonist, caused a concentration-dependent inhibition of electrically-evoked contractions (Figure 3a,b) which was abolished by 1 μM naloxone (data not shown). Ibogaine (30 μM) and 18-methoxycoronaridine (30 μM) caused a 3 – 5 fold parallel rightward displacement of the inhibitory effect of DAMGO (Table 1, Figure 3a,b). As indicated above, ibogaine and 18-methoxycoronaridine exerted pronounced effects on the neurogenic contraction, which raises the possibility that the effect observed against responses to DAMGO could simply arise from functional antagonism, rather than a direct interaction with prejunctional μ-opioid receptors. Since Sweetman *et al.* (1995) and Deecher *et al.* (1992) have reported that ibogaine is devoid of affinity for α_2-adrenoceptors ($pK_i<4$), we also examined the effect of ibogaine against responses to UK-14304 (a selective alpha$_2$-adrenoceptor agonist). As shown in Figure 3c, UK-14304 caused a concentration-dependent inhibition of electrically-evoked contractions of the rat isolated vas deferens. Ibogaine (30 μM) caused a 3 fold rightward, displacement of responses to UK-14304 (Table 1).

Guinea-pig urinary bladder

In the guinea-pig isolated urinary bladder electrical stimulation (10 Hz, 2 s) produced a contraction (2.5 ± 0.3 g. wt., $n=6$) which was significantly reduced by 0.1 μM atropine (Figure 4a,b), 300 μM suramin (Figure 4a,b) and abolished by a combination of both agents (data not shown), indicating the involvement of acetylcholine and ATP as neurotransmitters. Ibogaine (10 μM) and 18-methoxycoronaridine (10 μM) significantly enhanced electrically-evoked contractions in the absence and in the presence of 0.1 μM atropine, but failed to alter responses in the presence of 300 μM suramin (Figure 4; Table 2).

ATP (500 μM) elicited transient, reproducible contractions of the guinea-pig isolated bladder equivalent to $19.7\pm2.0\%$ of the contraction to 60 mM KCl (5.85 ± 0.87 g wt., $n=12$). As shown in Table 2, responses to ATP were not significantly affected by either ibogaine (10 μM) or 18-methoxycoronaridine (10 μM). It should be noted, however, that in three of the six preparations exposed to 10 μM ibogaine the contraction to 500 μM ATP increased by more than 50%; no change in response was noted in the remaining preparations.

Rat isolated portal vein

At rest, longitudinal segments of the rat isolated portal vein contracted spontaneously (0.34 ± 0.05 g wt., $n=9$). As shown in Figure 5, ibogaine (1 – 30 μM) caused a concentration-dependent increase in the magnitude of the spontaneous

Table 1 The effect of ibogaine and 18-methoxycoronaridine (18-MC) against prejunctional regulation of electrically-evoked contractions of the rat isolated vas deferens

	Agonist Potency (pD_2)	
	DAMGO	*UK-14304*
Control	6.71 ± 0.17	9.49 ± 0.13
30 μM Ibogaine	6.10 ± 0.15*	8.86 ± 0.08*
Control	6.87 ± 0.09	not attempted
30 μM 18-MC	6.18 ± 0.07*	–

Agonist potency (pD_2) has been shown as the mean $\pm$ s.e.mean of 5 – 6 observations. *denotes a statistically significant difference ($P<<0.05$; paired Student's *t*-test).

contractions which was also associated with a reduction in the frequency of the contractions (see also Figure 6). In marked contrast, 18-methoxycoronaridine (1–30 μM) failed to significantly increase the magnitude of spontaneous contractions (Figure 5 and 6).

Discussion

To date much of the research on the antiaddictive properties of ibogaine and related analogues has involved either radioligand binding studies using central nervous tissue or behavioural and microdialysis experiments on conscious rats and mice (see: Popik *et al*., 1995a). With the exception of the investigation by Miller & Godfraind (1983), in which the effects of tabernanthine and congeners were examined on isolated blood vessels, there have been no recent studies on smooth muscle

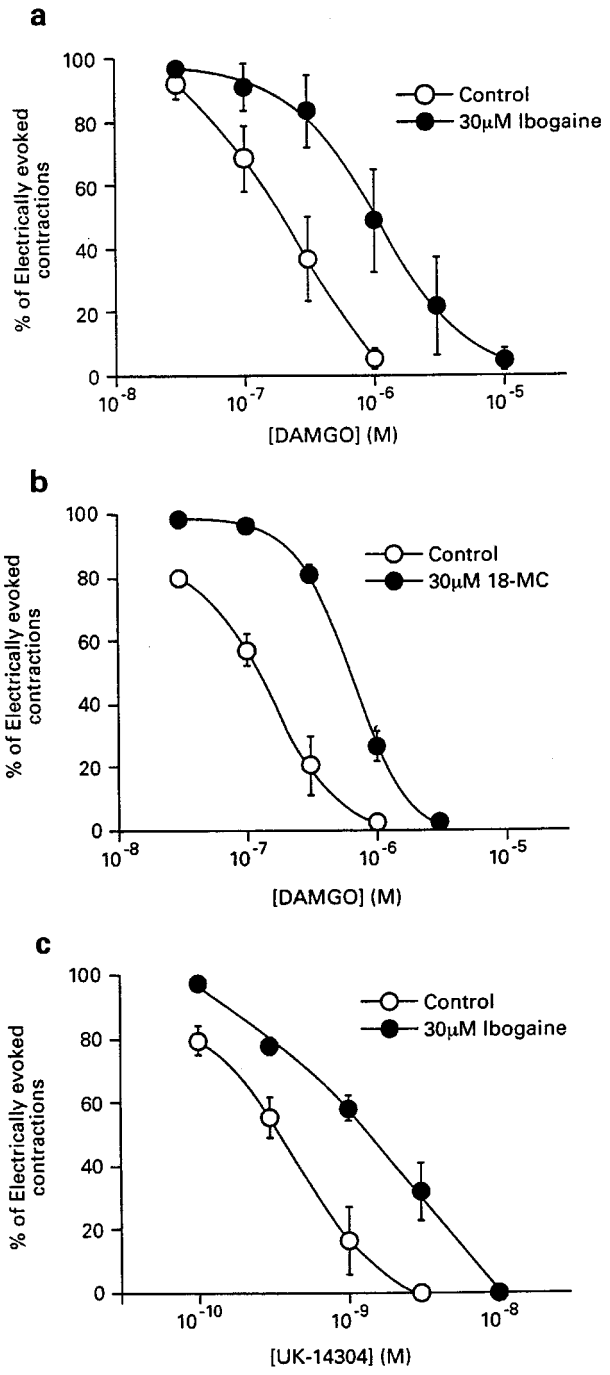

Figure 3 A graph of the effect of (a) 30 μM ibogaine, (b) 30 μM 18-methoxycoronaridine and (c) 30 μM ibogaine against (a,b) DAMGO-induced and (c) UK-14304-induced inhibition of electrically-evoked (0.1 Hz) contractions of the rat isolated vas deferens. Responses have been expressed as a percentage of the control response and are shown as the mean±s.e.mean of 5–6 observations.

Table 2 The effect of 18-methoxycoronaridine (18-MC) and ibogaine on the magnitude of electrically-evoked and ATP-induced contractions of the guinea-pig urinary bladder

	Ibogaine (10 μM)	*18-MC* (10 μM)
Electrically-evoked contractions		
(Control)	132.8±11.3*	124.3±7.7*
(Suramin (300 μM)	106.7±6.2	111.1±9.0
(Atropine (0.1 μM)	195.7±13.0**	227.1±26.3**
ATP-induced (500 μM) contractions	138.2±15.5	106.6±10.4

Responses in the presence of ibogaine and 18-methoxycoronaridine are expressed as a percentage of the contractions prior to the additions of the drugs (basal response = 100%) and are shown as the mean±s.e.mean of the 5–6 observations. *Denotes a statistically significant increase in the contractions (Student's unpaired *t*-test $P<0.05$); ** denotes a statistically significant increase in the contractions (Student's unpaired *t*-test $P<0.01$).

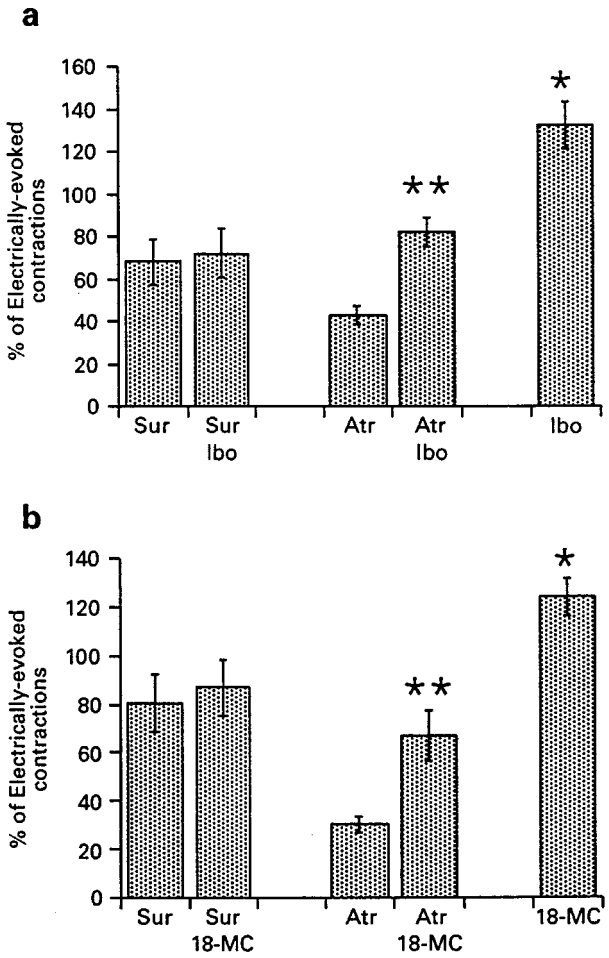

Figure 4 A graph of the effect of (a) 10 μM ibogaine (Ibo) and (b) 10 μM 18-methoxycoronaridine (18-MC) against electrically-evoked (10 Hz, 2 s trains every 5 min) contractions of the guinea-pig isolated urinary bladder in the absence and presence of either 0.1 μM atropine (Atr) or 300 μM suramin (Sur). Responses have been expressed as a percentage of the control response and are shown as the mean± s.e.mean of 5–6 observations.

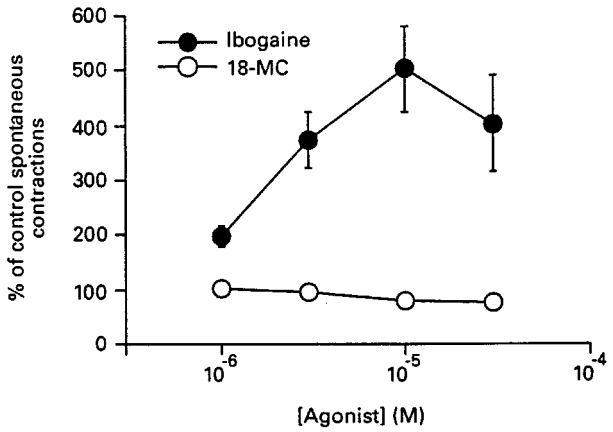

Figure 5 A graph of the effect of ibogaine and 18-methoxycoronaridine on spontaneous contractions of the rat isolated portal vein. The amplitude of contractions have been expressed relative to the contractions prior to the addition of the drugs and are shown as the mean ± s.e.mean of 4–5 observations.

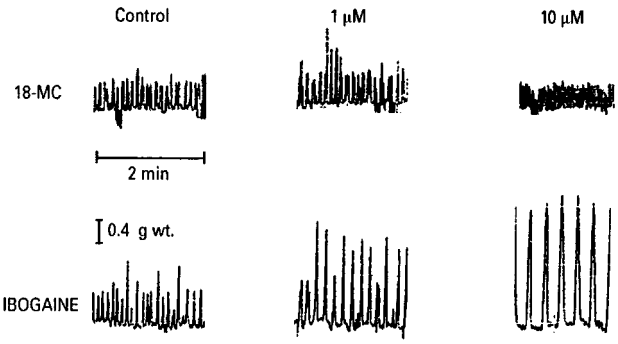

Figure 6 Representative trace recordings of the effect of ibogaine and 18-methoxycoronaridine (18-MC) on spontaneous contractions of the rat isolated portal vein. Note that only ibogaine increased the amplitude of the spontaneous contractions.

preparations. Our study on the effect of ibogaine and 18-methoxycoronaridine on isolated smooth muscle preparations from the rat and guinea-pig has provided valuable insights into properties of these compounds.

Considerable evidence has accumulated to suggest that μ-opioid receptors present an important target for the antiaddictive action of ibogaine (Deecher *et al*., 1992; Codd, 1995; Pearl *et al*., 1995; Sweetman *et al*., 1995; Rabin & Winter, 1996; Bagal *et al*., 1996). However, two observations in this study argue against a significant role of μ-opioid receptors in the peripheral actions of these compounds. First, although ibogaine and 18-methoxycoronaridine inhibited electrically-evoked contractions of the guinea-pig isolated ileum, an action consistent with activation of μ-opioid receptors (Miller *et al*., 1986; Leff & Dougall, 1988), this effect was not altered by the selective opioid receptor antagonist naloxone. Since naloxone does not discriminate between μ-opioid and κ-opioid receptors, both of which are present on the myenteric plexus of the guinea-pig ileum (Leff & Dougall, 1988), neither receptor can be implicated in the inhibitory effect of these compounds. Second, neither ibogaine nor 18-methoxycoronaridine mimicked the effect of DAMGO, a selective μ-opioid receptor agonist (Handa *et al*., 1981), in inhibiting electrically-evoked contractions of the rat isolated vas deferens. Rather, both agents caused a 2 fold increase in the neurogenic contractions. While a high concentration of each compound caused a rightward displacement of the DAMGO concentration response curve (an action consistent with blockade of prejunctional μ-opioid receptors), similar effects were also observed against α_2-adrenoceptor-mediated inhibition of electrically-evoked contractions. Since ibogaine (and presumably the congener 18-methoxycoronaridine) has no significant affinity for α_2-adrenoceptor binding sites (Deecher *et al*., 1992; Sweetman *et al*., 1995), functional antagonism, arising from the enhancement of electrically-evoked contractions, appears to be the most likely explanation for these effects. Thus, while we have no data from isolated smooth muscle preparations that either agent exerts a direct effect on the μ-opioid receptor, the possibility remains that active metabolites, e.g. noribogaine (see: Pablo & Mash, 1998; Glick *et al*., 1996b), may act at this site.

In the absence of an effect on μ-opioid receptors, the action of ibogaine and 18-methoxycoronaridine requires further comment. In the case of the guinea-pig ileum, a direct interaction with postjunctional muscarinic receptors seems unlikely since both agents were at least five times more potent at inhibiting the electrically-evoked contractions than at central muscarinic binding sites. Furthermore, the low potency of these compounds at central muscarinic binding sites is consistent with that reported for harmaline derivatives (β-carboline compounds structurally-related to ibogaine and 18-methoxycoronaridine) at guinea-pig myenteric plexus muscarinic binding sites labelled by [^{3}H]-QNB (Hider *et al*., 1981). This view is also supported by the failure of ibogaine and 18-methoxycoronaridine to significantly affect cholinergic contractions of the guinea-pig isolated urinary bladder (see below). Thus, at present the mechanism of action underlying the inhibitory effect of these compounds is unclear but it is noteworthy that Hider and colleagues (1981) described a non-competitive action for harmaline derivatives against acetylcholine-induced contractions of the guinea-pig isolated ileum.

With regards to the enhancement of purinergic contractions of the vas deferens, experiments on the guinea-pig isolated urinary bladder revealed that both agents also potentiated the atropine-resistant component of the neurogenic contractions; a response abolished by the P2X-purinoceptor antagonist suramin (Dunn & Blakeley, 1988; Hoyle *et al*., 1990). Since neither ibogaine nor 18-methoxycoronaridine produced similar effects against the cholinergic (suramin-resistant) component of electrically-evoked contractions of the guinea-pig urinary bladder, and failed to potentiate the ATP-induced contractions, a selective prejunctional action on purinergic nerves is the most likely explanation. Presently, the significance of this novel property of ibogaine and 18-methoxycoronaridine to their antiaddictive action is not clear, but the possibility exists that it may be counterproductive in terms of their proposed therapeutic use. Excitatory P2X-purinoceptors are known to be located in the locus coeruleus (Nieber *et al*., 1997), a major site for the integration of autonomic responses, and over-activity of this region of the brain is a characteristic feature of naloxone-induced 'withdrawal' in opiate-dependent rats (Nestler, 1992).

The outcome of the above experiments highlights the similar pharmacological properties of ibogaine and 18-methoxycoronaridine in several isolated smooth muscle preparations. However, the recent interest generated by 18-methoxycoronaridine stems from experimental evidence indicating that it lacks the pronounced tremorigenic properties of ibogaine (Glick *et al*., 1994; 1996a). Ibogaine is known to interact with Na^+ channels with low micromolar affinity, which has prompted the suggestion that this interaction is the basis of the tremorigenic activity (Deecher *et al*., 1992;

Sweetman *et al*., 1995). Interestingly, Doggerell & Bishop (1996) have reported that veratridine, an activator of Na^+ channels (Honerjager, 1982), increased spontaneous contractions of the rat isolated portal vein, while Miller & Godfraind (1983) noted that tabernanthine (a tremorigenic congener of ibogaine; Glick *et al*., 1994) produced qualitatively similar effects. In the present study we found that ibogaine, but not 18-methoxycoronaridine, caused a concentration-dependent enhancement of spontaneous contractions of the portal vein. The lack of effect of 18-methoxycoronaridine, a non-tremorigenic ibogaine congener (Glick *et al*., 1996a), on spontaneous contractions of the portal vein suggests that this preparation may be useful as a screen to detect agents devoid of this undesirable activity. Also, these findings indicate that modulation of Na^+ influx is unlikely to account for the enhancement of purinergic contractions observed in either the rat vas deferens or guinea-pig urinary bladder.

In summary, using isolated smooth muscle preparations from the rat and guinea-pig we failed to detect any significant effect of either ibogaine or 18-methoxycoronaridine on prejunctional μ-opioid receptors regulating transmitter release. While both compounds exhibited a novel synergistic interaction with P2X-purinoceptors, differential effects on spontaneous contractions of the rat isolated portal vein were noted. The latter observation may be indicative of differences in their action at vascular Na^+ channels.

M.K. Mundey was supported by the BBSRC (ROPA Award).

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(Received July 29, 1999
Revised November 23, 1999
Accepted January 10, 2000)