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Activities of extract and constituents of *Banisteriopsis caapi* relevant to parkinsonism

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Abstract

Dopamine deficiency is characteristic of Parkinson's disease (PD) and treatments aim at elevating levels by administration of its precursor L-dihydroxyphenylalanine (L-DOPA), or inhibiting monoamine oxidases (MAOs), thus preventing its breakdown. Reports of improvements in PD patients treated with *Banisteriopsis caapi* extracts stimulated investigation of *B. caapi* stem extract and its two ingredients, harmine and harmaline for these activities.

Tests for MAO inhibition using liver homogenate showed that extract and harmaline showed a concentration-dependent inhibition of MAO A (IC_{50} 1.24 μg/ml and IC_{50} 4.54 nM, respectively) but had little effect on MAO B activity.

The extract at 2.5 mg/ml caused a highly significant increase in release of [^{3}H]dopamine from rat striatal slices, as did 200 μM harmine and 6 μM harmaline. In both these experiments, the amount of harmine present could not account for the total activity of the extract.

The ability of harmine and harmaline to stimulate dopamine release is a novel finding. These results give some basis to the reputed usefulness of *B. caapi* stem extract in the treatment of PD.

Keywords: *Banisteriopsis caapi*; Parkinson's disease; Indole alkaloid; Harmine; Harmaline; Dopamine release; Monoamine oxidase inhibition; MAOI

1. Introduction

Banisteriopsis caapi is a woody vine that grows in the Amazonian basin and is famous as an ingredient of the hallucinogenic drink known variously as ayahuasca (English), 'hoasca' (Brazil), 'yage' (Colombia) and 'caapi' (Schultes and Raffauf, 1990). Ayahuasca is nowadays used by some religious groups in Brazil to treat alcoholism, but its hallucinatory properties have been known to many of the indigenous peoples of the Amazon for centuries. Although the fine details of the hallucinatory mechanism are not known, it is generally accepted that *N*,*N*-dimethyltryptamine, from ingredients of the ayahuasca such as the leaves of *Psychotria* species, plays an important part in the hallucinogenic CNS activity although it has been suggested that harmaline **1** from the *B. caapi* component of the drink also contributes in its own right. Harmaline and the related compound harmine **2**, also present in *B. caapi*, also inhibit the activity of monoamine oxidases (MAOs) that otherwise would metabolise the *N*,*N*-dimethyltryptamine and prevent sufficient amounts reaching the CNS (Callaway et al., 1999), thus allowing the traditional oral administration to exert its effect.

In the 1920s and early 1930s, harmine, then called banisterine, was used in animals and patients with symptoms similar to PD (Sanchez-Ramos, 1991). Interest died quickly, but very recently, reports have described the beneficial use of an extract of *B. caapi* (Griseb.) Morton (Malpighiaceae) on patients with Parkinson's disease (PD) (Sanchez-Ramos, 1991; Serrano-Dueñas et al., 2001). PD is known to be associated with low levels of dopamine in the substantia nigra of the basal ganglia in the brain and standard therapy consists in giving L-dihydroxyphenylala-

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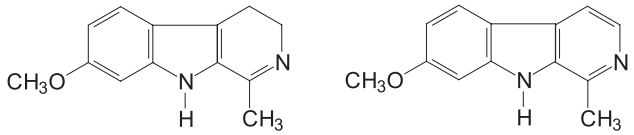

1 Harmaline **2** Harmine

Fig. 1. Alkaloids present in *B. caapi* bark.

nine (L-DOPA), which crosses the blood–brain barrier and is converted into dopamine in the brain. Although, in the short term, L-DOPA is effective in controlling the symptoms of PD, it often results in unpredictability and involuntary movements so other therapeutic agents are being sought. Approaches to compounds having a similar net effect may involve compounds acting as dopamine agonists, those elevating dopamine levels by inhibition of MAOs and those that stimulate the release of dopamine from cells where they are produced or stored in the brain.

In view of the promising results obtained in initial clinical studies, it was decided to investigate the activity in vitro relevant to PD of an extract of *B. caapi* stem and two of the major alkaloids present, harmine **2** and harmaline **1** (Fig. 1).

2. Methods

2.1. Plant material and extraction

Dried stem of *B. caapi* was obtained from Dr. Marcos Serrano-Duenas, Neurology Service, Carlos Andrade Marin Hospital Quito, who had made personal observations relayed to one of us (A.D. Lees) on the beneficial effects of aqueous extracts of this material on PD patients.

The stem was ground into a powder and 50 g was extracted by refluxing with 500 ml distilled water for 1 h. This approximates to the method of preparation of the extract used in the clinic in Ecuador. After filtration, the plant material was extracted with a second amount of water in the same way. The two filtrates were combined and freeze-dried to yield 10.7 g of extract.

2.2. Thin-layer chromatographic analysis of the extract

The freeze-dried extract (100 mg) was dissolved in 5.0 ml of methanol. Ten microliters of this solution were applied to TLC plate. Harmine 2 mg/ml and harmaline 2 mg/ml were applied as reference solutions in a series of volumes from 1 to 10 μl to a precoated silica gel plate (Merck 60 GF_{254}) and the plate developed using the mobile phase chloroform:methanol:water (64:50:10). Zones produced were visualised by spraying with Dragendorff's reagent and the intensity of each zone measured by reflectance scanning using a Shimadzu TLC scanner at 500 nm. A straight-line calibration plot was produced using the densitometer reading against amount over the range straddling the intensity of the relevant zone given for the compound by the extract. From this plot, the amount of harmine present in the 200 μg of extract applied was found to be 3.8 μg, i.e., 1.9% wt/wt in the lyophilised extract and 0.41% wt/wt in the dried root. By the same criteria, 0.22% wt/wt harmaline was found in the lyophilised extract and 0.047 wt/wt in the dried root.

2.3. Material for biological tests

Male Wistar rats (190 ± 10 g) were killed by stunning and decapitation. The abdomen was opened and a liver sample was dissected. The liver was homogenised in 20 volumes (5% wt/vol) of ice-cold sodium phosphate buffered 0.9% saline (PBS; 0.1 M, pH 7.4). Homogenates were stored at − 70 °C until assayed for MAO activity. On the day of the assay, samples were allowed to defrost on ice and were mixed by vortexing. Liver samples were thawed and then further diluted 1:10 with ice-cold PBS and vortexed to produce a final liver homogenate of 1:200 volumes (0.5% wt/vol).

2.4. MAO inhibition assay

2.4.1. In vitro assay

An in vitro assay was designed to measure the effects of the plant extract and compounds on MAO A and MAO B activity. *B. caapi* extract (10^{-15} to 10^{-5} g/ml), harmine **2** (10^{-15} to 10^{-5} M), clorgyline (10^{-15} to 10^{-5} M) and selegiline (10^{-15} to 10^{-5} M), were investigated on MAO A and MAO B activity in rat liver in vitro. The assay was performed as described below following preincubation of *B. caapi* extract, harmine, clorgyline and selegiline, dissolved in PBS (0.1 M, pH 7.4) with the liver homogenate for 30 min at 37 °C. Nonenzymatic formation of [^{14}C]5-HIAA or [^{14}C]5-PAA was determined by replacing the enzyme with PBS and performing the assay as described above. In controls, inhibitor was replaced by PBS (0.1 M, pH 7.4).

MAO activity was assessed by a modification of the method of Fowler and Orlando (1980). Aliquots (20 μl) of liver homogenate (0.5% wt/vol final concentration) were added to 20 μl of the appropriate substrate (1 μM [^{14}C]5-HT, Amersham, UK; specific activity 57 mCi/mmol for MAO A or 0.25 μM [^{14}C]phenylethylamine (PEA), Amersham, UK; specific activity 52 mCi/mmol for MAO B) dissolved in PBS (0.1 M, pH 7.4) in a final volume of 250 μl, and incubated for 30 min at 37 °C. The assay was terminated by the addition of 200 μl of 2 M citric acid. All stages of the assay up to the acid activation, with the exception of the incubation periods, were carried out at 0 °C.

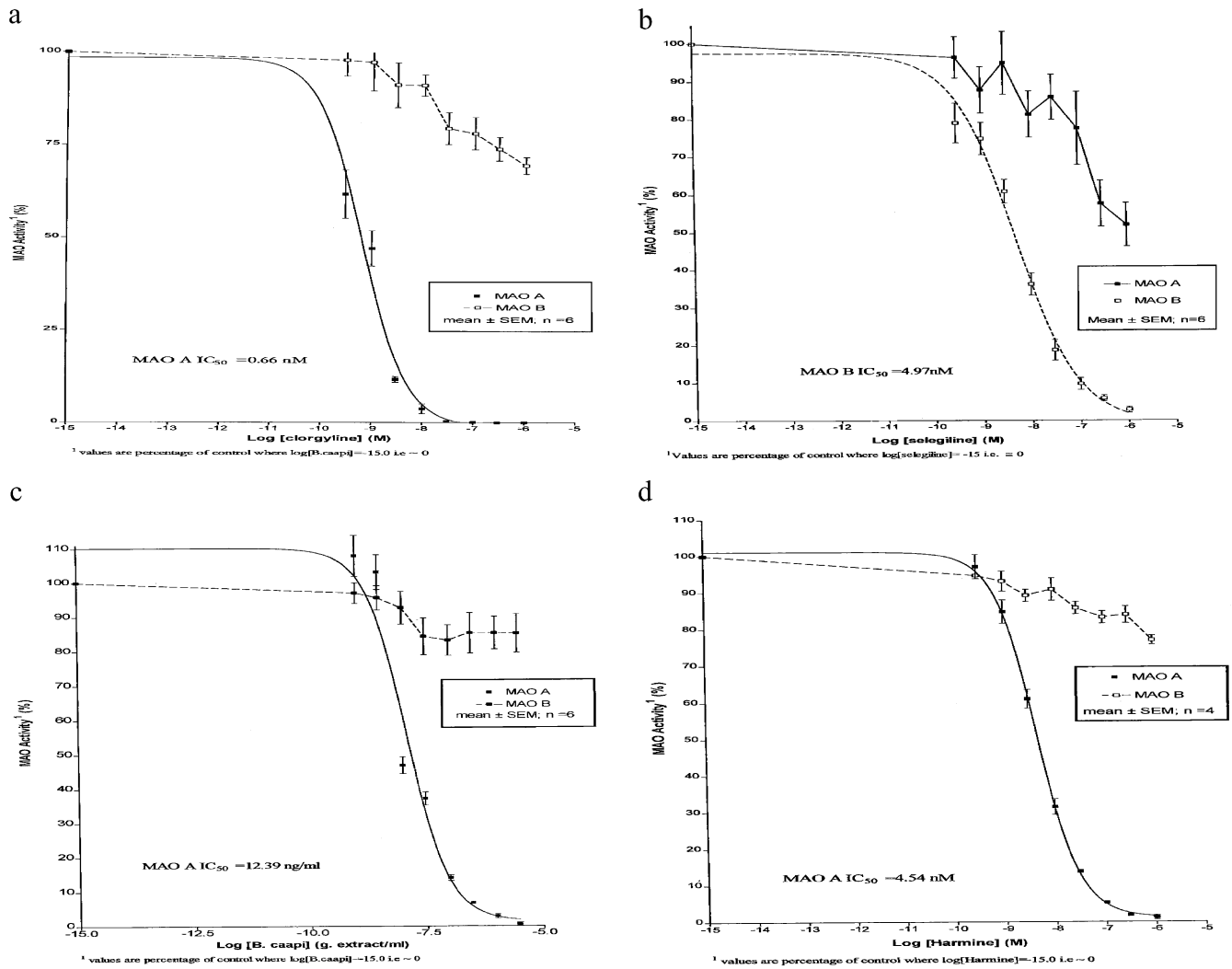

Fig. 2. (a) Inhibition of MAO isoforms by clorgyline. (b) Inhibition of MAO isoforms by selegiline. (c) Inhibition of MAO isoforms by *B. caapi* extract. (d) Inhibition of MAO isoforms by harmine.

2.4.2. Extraction of deaminated metabolites

For determination of MAO A activity, the deaminated metabolite formed from 5-HT [5-hydroxyindoleacetic acid (5-HIAA)] was extracted into ethyl acetate:toluene (1:1 vol/vol, saturated with water). For determination of MAO B activity, the deaminated metabolite formed from PEA [phenylacetic acid (PAA)] was extracted into toluene. The respective solvent (1 ml) was added to the deactivated reaction mixture and samples were vortexed for 30 s and then centrifuged at 2000 rpm at 4 °C for 5 min. The lipophilic phase, containing the deaminated metabolite, was carefully extracted (0.85 ml) and placed into polyethylene vials and liquid scintillation cocktail (Optiphase 'HIsafe' 2; Fisher, UK; 3 ml) was added. The radioactivity in each sample was determined by liquid scintillation spectroscopy (Packard 2425 liquid scintillation spectrometer) at an efficiency of 95%. The mean extraction efficiencies were 54% and 62% for 5-HIAA and PAA, respectively.

2.4.3. Drugs

The radioactive substrates 5-hydroxytryptamine-[side chain-2-^{14}C]creatine sulphate ([^{14}C]5-HT) and β-phenylethylamine-[side chain-ethyl-1-^{14}C]hydrochloride ([^{14}C]PEA) were obtained from the Radiochemical Centre, Amersham. All standard compounds were obtained from Sigma. All other reagents were standard laboratory reagents of analytical grade wherever possible.

Table 1
Inhibition of rat liver MAO isoforms by positive controls, *B. caapi* extract and harmine

Compound	IC_{50} MAO-A	IC_{50} MAO-B
Clorgyline	6.6×10^{-10} M	$>10^{-6}$ M
Selegiline	$>10^{-6}$ M	4.97×10^{-9} M
B. caapi extract	1.24×10^{-8} g/ml	$>10^{-5}$ g/ml
Harmine	4.54×10^{-9} M	$>10^{-6}$ M

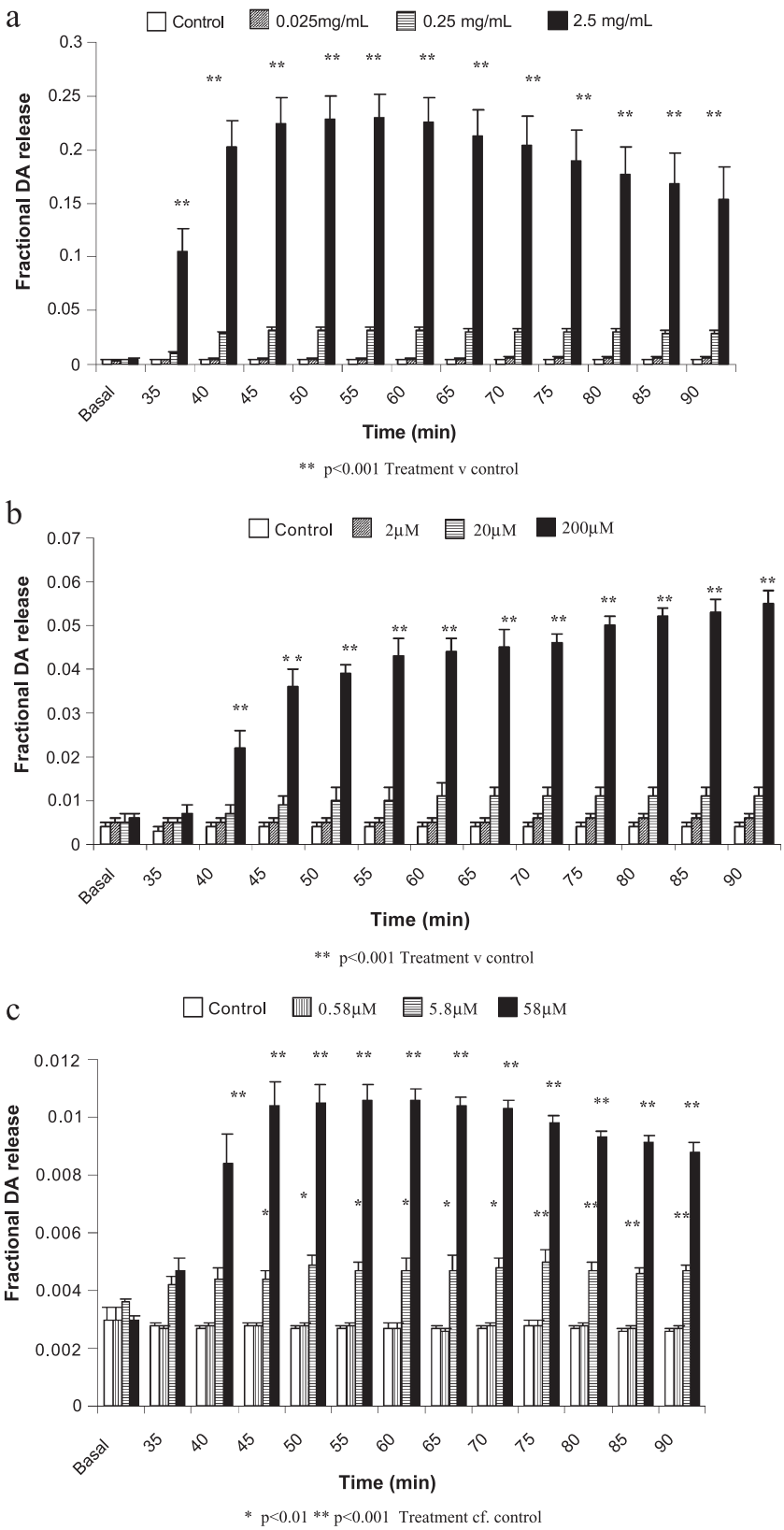

Fig. 3. (a) Effect of *B. caapi* extract on striatal DA release. (b) Effect of harmine on striatal DA release. (c) Effect of harmaline on striatal DA release. * $P < .01$; * * $P < .001$, treatment vs. control.

2.4.4. Data and statistical analysis

Enzyme activity was calculated from the values of radioactive dissolutions per minute to moles product formed per milligram tissue per 30 min. Activity was presented as percent control (no inhibitor). IC_{50} values were calculated from plots of inhibitor concentration against percent inhibition of MAO activity using Prism 3 Software (Graphpad Software). Each assay was performed in triplicate on four to six separate homogenates.

2.5. In vitro release of [^{3}H]dopamine ([^{3}H]DA) from rat striatal slices

Male Wistar rats (280–320 g) were killed by cervical dislocation and decapitation, their brains removed and immediately cooled on ice. Paired striata were dissected and cut coronally into slices (300 μm) using a McIlwain tissue chopper. Striatal slices were washed twice with ice-cold Krebs buffer (in mM: NaCl, 138; KCl, 5; $NaHCO_3$, 10; $CaCl_2$, 1.2; NaH_2PO_4, 1; glucose, 10; pargyline, 0.025; ascorbic acid, 0.17, gassed with O_2/CO_2 95%/5%) and then placed into 4 ml Krebs at 37 °C. Slices were incubated in Krebs containing [^{3}H]DA (50 nM; [7,8-^{3}H]DA, 48 Ci/mmol; Amersham, UK) for 30 min at 37 °C. The incubation was terminated by washing three times with ice-cold Krebs. Two to three slices were placed into each of eight superfusion chambers and superfused with Krebs (250 μl/min at 37 °C) throughout the experimental period. Superfusate was collected every 5 min (1.25 μl) and counted for tritium by liquid scintillation spectroscopy (Tri-Carb 460 liquid scintillation system, Canberra Packard, UK) at an efficiency of 55%. At the end of the experiment, slices were recovered to determine the residual radioactivity. Fractional release was determined by expressing the [^{3}H]DA in each fraction of superfusate relative to the total [^{3}H]DA remaining in the slices at the beginning of that time point.

2.6. The effect of B. caapi extract, harmine and harmaline on [^{3}H]DA release from rat striatal slices

After superfusion for 60 min with Krebs to allow recovery from the preparation procedure, the first six fractions were used to determine basal [^{3}H]DA efflux. *B. caapi* extract, harmine or harmaline was then incorporated in the Krebs for 60 min such that each test compound was assessed in tissue from the same rat at each dose. Results are expressed as change in fractional dopamine release over 60 min. All experiments were performed in duplicate on four separate occasions.

Data were analysed for the effect of time and treatment by two-way ANOVA. If the effect was associated with a probability of less than 5%, further analysis was performed using a one-way ANOVA and Dunnett's posttest.

3. Results

3.1. MAO inhibition

Clorgyline (10^{-15} to 10^{-6} M) produced a concentration-related inhibition of MAO A activity with an IC_{50} of 0.66 nM. Clorgyline produced some inhibition of MAO B with maximal inhibition of 25% at 10^{-6} M, and an IC_{50} of greater than 10^{-6} M (Fig. 2a, Table 1). It is appropriate to quote an IC_{50} value since clorgyline is known to produce a reversible inhibition at these concentrations.

Selegiline (3×10^{-9} to 10^{-6} M) produced a concentrated-related inhibition of MAO B activity with an IC_{50} of 5.0 nM. Selegiline produced some inhibition of MAO A at higher concentrations, with a maximal inhibition of 48% at 10^{-6} M, and an IC_{50} of greater than 10^{-6} M (Fig. 2b, Table 1).

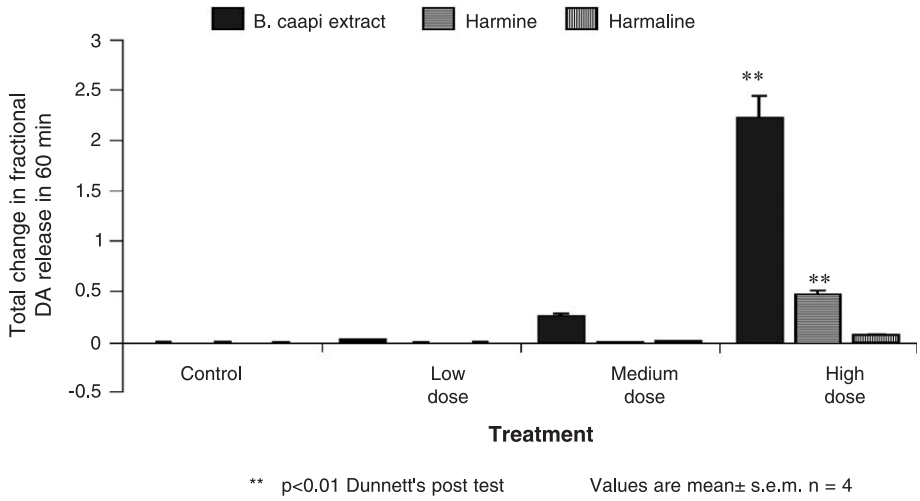

Fig. 4. Change in fractional DA release over 60 min due to plant extract and equivalent doses of harmine and harmaline. * * $P < .01$, Dunnett's posttest. Values are mean ± S.E.M. $n = 4$.

B. caapi extract also produced a concentration-related inhibition of MAO A activity with an IC_{50} of 1.24×10^{-6} g/ml. *B. caapi* extract had little effect on MAO B activity, producing a maximal inhibition of 15% at between 10^{-5} and 10^{-6} g/ml (Fig. 2c, Table 1).

Harmine (3×10^{-9} to 10^{-6} M) produced a concentration-related inhibition of MAO A activity with an IC_{50} of 4.54×10^{-9} M. Harmine had little effect on MAO B activity, with maximal inhibition of 20% at 10^{-6} M (Fig. 2d, Table 1).

3.2. Release of [^{3}H]DA from rat striatal slices

The results of these experiments are shown in Fig. 3a, b and c. Fig. 4 shows the change in fractional DA release over 60 min for the extract and the two compounds. Superfusion with the *B. caapi* extract (2.5 mg/ml) produced an immediate and substantial increase in [^{3}H]DA release compared to control. The lower concentration (0.25 mg/ml) produced a strong trend that did not reach statistical significance.

Harmine (200 μM) produced a significant increase in [^{3}H]DA release but not at the lower concentrations. In comparison, harmaline increased [^{3}H]DA release, not only at the highest concentration (58.3 μM) but also at 5.83 μM, although the total amount of [^{3}H]DA released by harmaline was much less than with harmine (Fig. 4).

4. Discussion

4.1. Content of harmine in extract

The value of 19.2 mg harmine per gram lyophilised extract is comparable with values obtained in a previous study on several samples where the mean value was found to be 23.8 mg/g (McKenna et al., 1984).

4.2. MAO inhibition

It can be clearly seen from Fig. 2a–d that the *B. caapi* extract and harmine inhibit MAO-A rather than MAO-B, i.e., they behave like clorgyline rather than selegiline. It is reasonable to assume that the activity of the extract was due primarily to harmine. The MAO-A inhibitory effect for harmine reported in this study (IC_{50} 4.54 nM) is in fairly close accordance with that reported by McKenna et al. (1984) who found the IC_{50} value to be 12.6 nM. Insufficient harmaline was available to test its activity but a previous study has shown that it had much the same activity as harmine (McKenna et al., 1984). In terms of equivalent concentration of harmine, the IC_{50} of the extract corresponds to only 0.0011 nM harmine, which is at least two orders of magnitude less than harmine alone (4.54 nM). Even allowing for an additive contribution from the harmaline present, this value implies that the activity of the extract is much greater than that of the equivalent dose of harmine. A synergistic effect is therefore implicated or the existence of a much more active compound that has yet to be isolated.

4.3. Release of DA from striatal slices

B. caapi extract, harmine and harmaline produced an increase in [^{3}H]DA release from rat striatal slices. Fractional DA release increased immediately for *B. caapi* extract and harmine but harmaline showed some delay. This may reflect a delayed action or may be an artefact of small sample size. Harmaline was more potent than harmine as 5.83 μM significantly increased [^{3}H]DA release, whereas a higher concentration of harmine 20.1 μM did not. However, it should be noted that harmine produced considerably greater total release following 200 μM than harmaline at 50 μM. The difference between the concentration giving an effect for the alkaloids alone and that of the equivalent concentration in the extract is again noteworthy. Visual observation of the TLC plate confirmed that the ratio of harmine to harmaline in the extract was about 5:1, much the same as that reported by McKenna et al. (1984). For the 2.5-mg/ml solution, which gave a significant effect, this would give concentrations of harmine and harmaline of 20.1 and 5.83 μM, respectively, the middle concentration of the alkaloids used. It can be seen from Fig. 4 that this concentration of harmine did not significantly increase the release of DA although there was some increase at the middle concentration of harmaline, but not as great as that given by the extract. Thus, again the extract produced a greater effect than that expected from the content of the two major alkaloids and the possibility of synergism or the presence of a more active compound that was not isolated is again implied.

An additional mechanism for dopamine release might be the activation of specific β-carboline receptors. The β-carboline harman, which is closely related in chemical structure to harmine and harmaline, has been shown to stimulate dopamine release in tissue that is rich in these receptors (Baum and Hill, 1996).

4.4. Overall conclusions

The ability of the extract to inhibit MAO A would have the net effect of raising DA levels in the CNS, and thus alleviating symptoms of Parkinson's disease if they are able to cross into the bloodstream from the intestine and also cross the blood–brain barrier. In addition, the novel activity reported here for the extract in being able to stimulate release of DA from striatal cells would also increase DA levels and have the same overall beneficial effect in Parkinsonism. The recently reported clinical study (Serrano-Dueñas et al., 2001), which showed that *B. caapi* stem extract gave improvements in patients suffering from Parkinson's disease, indicates that the alkaloids may in fact be able to reach the CNS. However, it is plain that more work needs to be carried out to investigate the possible presence

of more active compounds or of synergism. Studies also need to be performed in the longer term to elucidate whether prolonged dosing with the extracts or the alkaloids results in the dyskinesia associated with L-DOPA therapy.

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