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Research article

Harmane: An atypical neurotransmitter?

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HIGHLIGHTS

- [^{3}H]harmane accumulation was overt in rat brain cortex at 37 °C.
- Uptake was unaltered by monoamine uptake blockers or imidazoline receptor ligands.
- [^{3}H]harmane transport was temperature-insensitive and Na^+-independent.
- The release of preloaded [^{3}H]harmane was not evoked by 25 mM K^+ from rat brain.

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ABSTRACT

Harmane is an active component of clonidine displacing substance and a candidate endogenous ligand for imidazoline binding sites. The neurochemistry of tritiated harmane was investigated in the present study examining its uptake and release properties in the rat brain central nervous system (CNS) *in vitro*. At physiological temperature, [^{3}H]harmane was shown to be taken up in rat brain cortex. Further investigations demonstrated that treatment with monoamine uptake blockers (citalopram, nomifensine and nisoxetine) did not alter [^{3}H]harmane uptake implicating that the route of [^{3}H]harmane transport was distinct from the monoamine uptake systems. Furthermore, imidazoline ligands (rilmenidine, efaroxan, 2-BFI and idazoxan) showed no prominent effect on [^{3}H]harmane uptake suggesting the lack of involvement of imidazoline binding sites. Subsequent analyses showed that disruption of the Na^+ gradient using ouabain or choline chloride did not block [^{3}H]harmane uptake suggesting a Na^+-independent transport mechanism. Moreover, higher temperatures (50 °C) failed to impede [^{3}H]harmane uptake implying a non-physiological transporter. The failure of potassium to evoke the release of preloaded [^{3}H]harmane from rat brain cortex indicates that the properties of this putative endogenous ligand for imidazoline binding sites do not resemble that of a conventional neurotransmitter.

1. Introduction

The family of β-carbolines has drawn great interest over the years due to their natural existence in plants and animals and their diverse effects on biological systems. These compounds are formed endogenously *via* a Pictet–Spengler reaction between indolealkylamines and aldehydes [1]. The aromatic β-carbolines, harmane and norharmane, are formed *via* the oxidation of their tetrahydro-β-carboline (THβC) precursor by heme peroxidases [2]. Furthermore, the metabolic fate of these β-carbolines were shown to be driven by subtypes of cytochrome P450 enzymes [3]. β-Carbolines are reported to interact with different classes of receptors, namely serotonin, dopamine, benzodiazepine, opiate, nicotine, histamine as well as a novel class of receptors/binding sites termed the imidazoline binding sites (I-BS) [4–8]. The existence of a heterogenous family of binding sites that bind imidazoline compounds has drawn great attention in identifying an endogenous ligand. The first potential candidate discovered in rat and bovine brain was clonidine-displacing substance (CDS) [9]. CDS was shown to displace [^{3}H]clonidine with high affinity in bovine cerebral cortex [9]. Further evaluations showed that CDS not only interacts with α_2-adrenoceptors (α_2-AR) but in addition displays affinity at I-BS [10]. Soon after the discovery of CDS, agmatine was postulated to be an endogenous substrate at I-BS. It shared similar

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Table 1
Effect of monoamine uptake blockers on [^{3}H]harmane uptake in rat brain cortex.

Monoamine uptake blockers	[^{3}H]Harmane uptake (fmol/mg/20 mins)		
	Control	+Treatment	*P* value
Nisoxetine (1 μM)	880.8 ± 193	1369 ± 376	0.271
Nomifensine (10 μM)	1070 ± 339	1128 ± 329	0.904
Citalopram (10 μM)	801.3 ± 257	703.4 ± 177	0.76

Specific uptake was defined as uptake at 37 °C minus uptake at 0 °C. The above data represent the mean ± s.e.mean (vertical bars) of 5–7 independent experiments each performed in triplicate. Statistical analyses were carried out using Student's unpaired *t*-test (control *vs*. treatment). Significance was set at $P < 0.05$.

binding properties to CDS, binding to α_2-AR and the two subclasses of I-BS (I_1 and I_2), albeit weakly [11]. However, CDS and agmatine are two separate entities as their molecular weights [11] and distribution [12] differ considerably. Work from our laboratory has identified the active components of CDS from bovine lung to be harmane and harmalan [13], exhibiting marked nanomolar affinity at I_1-BS and I_2-BS [14].

Harmane has been shown to elicit numerous effects similar to those of I-BS selective ligands. In rat brain, harmane was reported to regulate blood pressure in a similar pattern of response to I_1-BS ligands [15,16]. More recently, harmane has been shown to augment the antinociceptive effect induced by nicotine and ethanol in the tail flick test, and such an effect was reproduced by I_1-BS and I_2-BS selective ligands [17]. Harmane has also been reported to exhibit a number of physiological and psychological effects attributable to I_2-BS involvement; such as monoamine turnover [18], hyperphagia [19], mood [20], drug discrimination and withdrawal [21]. Lastly, harmane displays insulin secretagogue properties in pancreatic β-cells which is a common functional trait of the atypical I_3-BS [22,23].

Taken together the above findings it is evident that harmane is a functional ligand at all three I-BS and as such a potential endogenous candidate at I-BS. The aim of the current study was to determine whether harmane displays properties that resemble that of classical neurotransmitters examining its potential uptake and release in rat brain *in vitro*.

2. Methods and materials

2.1. Preparation of brain tissue slices

Male Wistar rats (250–275 g) were killed by stunning followed by decapitation and brains were immediately removed. Brain tissue slices were prepared according to the methods of Abu Ghazaleh et al. (2004) [24].

2.2. Uptake studies

Uptake was evaluated by incubating cortical tissue slices in Krebs buffer containing 50 nM [^{3}H]harmane, [^{3}H]serotonin (5-HT), [^{3}H]dopamine (DA) or [^{3}H]noradrenaline (NA) at physiological temperature (37 °C) for 20 min in the presence or absence of monoamine uptake blockers, I-BS ligands or omission of Na^+ from buffer medium with choline chloride/50 μM ouabain. Non-specific uptake was determined on ice (0 °C). Reaction was terminated by rapid filtration and tissue was washed in ice-cold Krebs buffer. Tritium tissue content was determined by scintillation counting and tissue protein contents were estimated using the Bradford method with bovine serum albumin as a standard [25].

2.3. Neurotransmitter release assays

Brain cortical tissue slices were preincubated with 100 nM [^{3}H]harmane, [^{3}H]DA, [^{3}H]NA or [^{3}H]5-HT for 20 min at 37 °C. Washed slices were loaded into a multi-chamber Brandel superfusion apparatus and perfused at 37 °C with oxygenated Krebs solution (0.4 ml/min) containing 0.5 mM ascorbate and 0.35 mM pargyline. Perfusate samples were collected at 4 min intervals. Stimulation of [^{3}H]radioligand release was evoked with 25 mM KCl at $t = 12$ (S_1), 40 (S_2), and 68 (S_3) minutes for a period of 2 min. The tritium content of the perfusate samples as well as that remaining in the tissue slices were determined by scintillation counting. Fractional efflux of the [^{3}H]radioligands was calculated by dividing the tritium overflow in each fraction perfusate by the amount of radioactivity in the tissue at the start of each particular collection. Data are expressed as fractional release to allow for neurotransmitter depletion.

In these experiments, efforts were made to maximise usage of animal tissue and minimise animal suffering. All animal euthanasia were carried out by Schedule 1 in compliance with the U.K. Animals (scientific procedures) Act 1986 and associated guidelines.

Table 2
Effect of imidazoline binding site ligands on [^{3}H]harmane uptake in rat brain cortex.

Imidazoline binding site ligands	[^{3}H]harmane uptake (fmol/mg/20 min)		
	Control	+Treatment	*P* value
I_1-BS ligands			
Rilmenidine (10 μM)	405.8 ± 75.7	402.8 ± 57.7	0.975
Rilmenidine (100 μM)	405.8 ± 75.7	405.86 ± 30.1	1.00
Efaroxan (10 μM)	409.9 ± 62.3	415.5 ± 52.6	0.947
Efaroxan (100 μM)	409.9. ± 62.3	401.2 ± 54.2	0.919
I_2-BS ligands			
Idazoxan (10 μM)	330.2 ± 36.1	349 ± 44.5	0.747
Idazoxan (100 μM)	330.2 ± 36.1	356.6 ± 40.8	0.638
2-BFI (10 μM)	282.8 ± 40.9	267.8 ± 46.9	0.814
2-BFI (100 μM)	282.8 ± 40.9	238.6 ± 40.9	0.462

Specific uptake was defined as uptake at 37 °C minus uptake at 0 °C. The above data represent the mean ± s.e.mean (vertical bars) of 4–6 independent experiments each performed in triplicate. Statistical analyses were carried out using Student's one-way ANOVA followed by Dunnett's *t*-test (control *vs.* treatment). Significance was set at $P < 0.05$.