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Planarians in pharmacology: parthenolide is a specific behavioral antagonist of cocaine in the planarian *Girardia tigrina*

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ABSTRACT Planarians are traditional animal models in developmental and regeneration biology. Recently, these organisms are arising as vertebrate-relevant animal models in neuropharmacology. Using an adaptation of published behavioral protocols, we have described the alleviation of cocaine-induced planarian seizure-like movements (pSLM) by a naturally-occurring sesquiterpene lactone, parthenolide. Interestingly, parthenolide does not prevent the expression of pSLM induced by amphetamines; in vertebrates, amphetamines interact with the same protein target as cocaine. Parthenolide is also unable to prevent pSLM elicited by the cholinergic compounds nicotine and cytosine or by the glutamatergic agents L- or D- glutamic acid or NMDA. Thus, we conclude that parthenolide is a specific anti-cocaine agent in this experimental organism.

KEY WORDS: *planaria, parthenolide, cocaine, amphetamine, seizures, behavior*

Introduction

Planarians are one of the best-characterized animal models in developmental biology and regeneration research (Newmark and Sánchez Alvarado, 2002) and are currently being rediscovered as a very useful animal model in neuropharmacology (Buttarelli *et al.*, 2008; Raffa and Rawls, 2008). Planarians are the first extant example of organisms displaying cephalization, including a primitive brain, sometimes referred to as cerebral ganglia, with many features in common with vertebrate nervous systems, such as multipolar neurons and dendritic spines (Sarnat and Netsky, 1985). Additionally, nearly every neurotransmitter found in mammals is present in planarians (Buttarelli *et al.*, 2008; Ribeiro *et al.*, 2005). One of the most interesting properties of this class of organisms is their remarkable capacity for regeneration, including the complete regeneration of their cerebral ganglia upon decapitation (Cebrià, 2007; Cebrià *et al.*, 2002; Gentile *et al.*, 2011). Working with this organism has several practical advantages. They are commercially available at very low cost and are very easily kept in the laboratory. No special equipment such as incubators, etc., is necessary. Additionally, the genome of a planarian species (*Schmidtea mediterranea*) has been sequenced (Robb *et al.*, 2008), which will undoubtedly help

to understand these organisms at the molecular level.

Two specific planarian behavioral endpoints have proven useful in pharmacological experiments: the observation of changes in motility (Raffa *et al.*, 2001) and the induction of planaria seizure-like movements (pSLM; Rawls *et al.*, 2011; Fig. 1A). Both responses are easily quantified and have been used to study the effect of a wide variety of psychoactive agents and abused drugs (Raffa and Rawls, 2008; Rawls, 2011).

Parthenolide (Fig. 1B) is the active component of the feverfew plant (*T. parthenium*), used to treat migraines. This compound has several known biochemical effects, including the inhibition of serotonin type II receptors (Weber *et al.*, 1997) and the enhancement of NF- κ B transcription factor function (Kang *et al.*, 2002). Also, parthenolide displays anti-inflammatory effects and inhibits I κ B kinases (Kwok *et al.*, 2001).

Parthenolide and related compounds have been shown (1) to prevent the expression of cocaine-induced behaviors in planarians, such as motility decrease and seizure-like hyperkinesias (Pagán

Abbreviations used in this paper: DAT, dopamine transporter; MAT, monoamine transporter; NET, norepinephrine transporter; NMDA, N-Methyl-D-Aspartate; pSLM, planarian seizure-like movements; SERT, serotonin transporter.

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et al., 2008) and (2) to prevent the expression of withdrawal-like behaviors induced by cocaine (Rowlands and Pagán, 2008). The lactone moiety of the parthenolide molecule is essential for its cocaine-antagonist properties (Pagán *et al.*, 2008). Further work established that the lactone moiety with a 4-methyl tail is sufficient to prevent the motility decrease induced by cocaine (Baker *et al.*, 2011). Significantly, similar results have been observed in vertebrates. Parthenolide alleviates the cocaine-induced effects on spontaneous firing of dopaminergic neurons in the ventral tegmental area of rats (Schwarz *et al.*, 2011).

Here we report that parthenolide prevents the pSLMs induced by cocaine but not the hyperkinesia induced by amphetamines, the cholinergic ligands nicotine and cytosine, or the glutamatergic ligands L- of D-glutamic acid and NMDA. These results suggest that parthenolide is a specific behavioral antagonist for cocaine in our experimental conditions.

Results

Fig. 1C shows that parthenolide up to a concentration of 50 μM does not induce significant pSLMs. This is consistent with previous reports, which show that at this concentration, parthenolide does not seem to produce any behavioral effects on the planarians (Pagán *et al.*, 2008; Rowlands and Pagán, 2008). Based on this, we used parthenolide at a concentration of 50 μM in all subsequent experiments. Fig. 1D shows that that 50 μM parthenolide prevents the

induction of cocaine-induced pSLM, as described before (Pagán *et al.*, 2008). Interestingly, parthenolide did not alleviate the behavioral response induced by amphetamines (Fig. 1E). However, please note that even though the global ANOVA p-values does not indicate a statistical difference between the worms exposed to amphetamine and the worms exposed to amphetamine/parthenolide in the whole curves, parthenolide significantly reduces the incidence of pSLM at the highest amphetamine concentration (10 mM). Also, parthenolide did not prevent the expression of pSLM in response to the cholinergic agents nicotine and cytosine (Fig. 2 A,B) or the glutamatergic ligands L- glutamic acid, D-glutamic acid or NMDA (Fig. 2 C,D,E).

Discussion

The negative results of parthenolide against all tested compounds except cocaine indicate a very important property of parthenolide, specificity. In clinical pharmacology, one of the main problems associated with medications are side effects, which are brought about by the interaction of a particular drug with multiple targets (Sheridan, 2003).

The accepted target for cocaine and amphetamines is the monoamine transporter (MAT) superfamily, which includes the dopamine transporter (DAT), the serotonin transporter (SERT) and the norepinephrine transporter (NET) (Torres *et al.*, 2003). The main function of the MATs is to regulate neurotransmitter

reuptake to the presynaptic terminal, hence regulating neurotransmission (Zahniser and Doolen, 2001). Neurotransmitter reuptake by specialized transporters was proposed as an inactivation mechanism as early as 50 years ago using radiolabeled noradrenaline (Hertting and Axelrod, 1961). The effect of cocaine in noradrenaline reuptake was also demonstrated using the same technique (Hertting *et al.*, 1961). Since then, this inactivation mechanism has been demonstrated in many neurotransmitter systems (Torres *et al.*, 2003). When abused drugs such as cocaine and amphetamine interact with the DAT, the dopamine concentration increases in the synaptic cleft; this increase causes a direct hyperactivation of postsynaptic dopamine receptors as well as extrasynaptic dopamine autoreceptors (Callier *et al.*, 2003). In fact, one of the main roles of the DAT seems to be the regulation of dopamine spillover to these autoreceptors (Cragg and Rice, 2004). In humans, the main target for cocaine and amphetamine is the DAT (Uhl *et al.*, 2002). Interestingly, DAT-knockout rodents are still sensitive to cocaine through increased participation of the NET and SERT in this process (Hall *et al.*, 2004).

In light of this information, our results showing that parthenolide prevented the behavioral effects of cocaine but not amphetamine was unexpected, since cocaine and amphetamines both interact with neurotransmitter transporters, albeit through different mechanisms. Cocaine

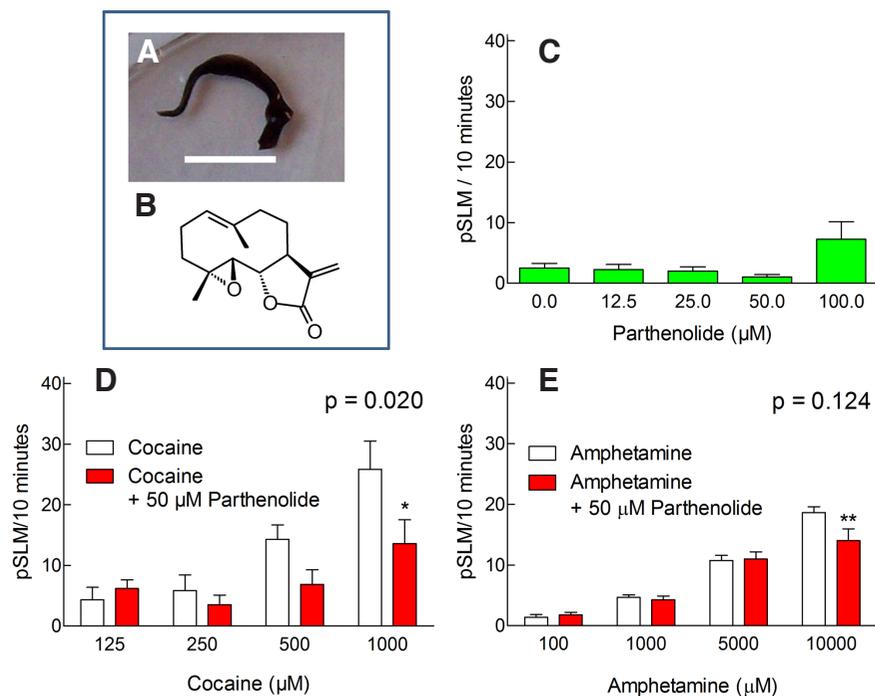


Fig. 1. Effect of parthenolide against cocaine and amphetamine. (A) Representative planarian seizure-like movement. The white bar is 1 cm long. (B) Parthenolide structure. (C) Parthenolide does not induce significant pSLMs. Each bar is the average of 4 worms. (D) Cocaine-induced pSLMs in the presence and in the absence of 50 μM parthenolide. (E) Amphetamine-induced pSLMs in the presence and in the absence of 50 μM parthenolide. Each bar is the average of 4-8 worms. The error bars represent the standard error of the mean. The number at the upper right corner of (D,E) represent the p-values generated by two-way ANOVA. The asterisks over some of the bars indicate significant difference between the column pairs ("*" < 0.05; "***" < 0.01) via a Bonferroni post-test.

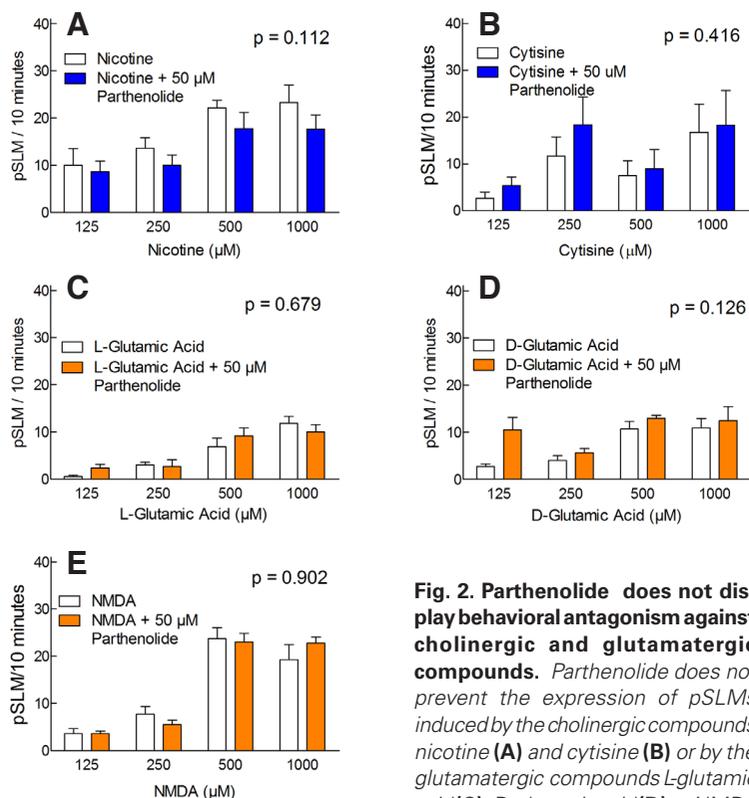


Fig. 2. Parthenolide does not display behavioral antagonism against cholinergic and glutamatergic compounds. Parthenolide does not prevent the expression of pSLMs induced by the cholinergic compounds nicotine (A) and cytisine (B) or by the glutamatergic compounds L-glutamic acid (C), D-glutamic acid (D) or NMDA

(E). The number at the upper right corner of each graph represent the *p*-values generated by two-way ANOVA. Each bar is the average of 5-10 worms. The error bars represent the standard error of the mean.

is a reuptake blocker, preventing the transport of the neurotransmitter back to the presynaptic site; amphetamine is considered a “releaser”, which acts as a false substrate of the transporter (Riddle *et al.*, 2005). In both cases, the net result is the abnormal increase of neurotransmitter molecules in the synaptic cleft, which accounts for the drug’s psychoactive properties (Iversen, 2006; Sager and Torres, 2011).

Using vertebrate pharmacology as a point of reference, our results suggests that parthenolide prevents the pSLM induced by cocaine by inhibiting an interaction with dopaminergic systems. However, this is not consistent with the amphetamine results (Fig. 1E) or by the fact that no significant pSLMs are detected when exposing the worms to a dopamine concentration of 1 mM (data not shown). Interestingly, preliminary results with another planarian species, *Dugesia dorotocephala*, seem to indicate that parthenolide alleviates amphetamine-induced pSLM (Rawls *et al.*, unpublished data). A possible interpretation is that in planarians, any catecholamine responses are modulated by compounds such as norepinephrine, octopamine or tyramine, as opposed to dopamine. Another implication of our results is that the pSLM are induced by cocaine/amphetamines and by cholinergic/glutamatergic compounds by interacting with different protein targets. This is amenable to pharmacological dissection of these distinct mechanisms.

In future experiments, we will study the effect of parthenolide against cocaine and amphetamine-like compounds in planarian behavior using paradigms such as conditioned place preference (Rawls *et al.*, 2011), cross-sensitization (Rawls *et al.*, 2010) and

withdrawal-like behavior (Sacavage *et al.*, 2008).

In a broader context, this work highlights the usefulness of planarians as an important animal model in pharmacology. In addition to the multiple advantages of using planarians described above, this model has been demonstrated to be relevant to mammalian pharmacology (Schwarz, 2011). Furthermore, since they can be studied all the way from molecular biology to behavior, these organisms will likely be developed as important tools in drug discovery research.

Materials and Methods

Girardia tigrina were purchased from Ward’s (Rochester, NY). The tested compounds were obtained from Sigma-Aldrich (St. Louis, MO) or Trocis (Ellisville, MO). General laboratory materials were purchased from Fisher Scientific (Suwanee, GA). All graphs and statistical procedures were done using the Prism software package (GraphPad Inc., La Jolla, CA). The experiments were carried out at room temperature using artificial pond water (APW, NaCl, 6 mM; NaHCO₃, 0.1 mM; CaCl₂, 0.6 mM). Dimethylsulfoxide (DMSO) at a concentration of 0.1% was present in all experimental solutions as a solubility-aiding agent for parthenolide. At this concentration, DMSO does not induce any observable behavioral or toxic effects in planarians (Pagán *et al.*, 2006).

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