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Rapid communication

Parthenolide prevents the expression of cocaine-induced withdrawal behavior in planarians

Amanda L. Rowlands, Oné R. Pagán *

Department of Biology, West Chester University, West Chester, PA, United States

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Abstract

We recently reported that parthenolide and related sesquiterpene lactones are able to prevent and reverse behavioral responses in planarian worms induced by acute cocaine exposure. Previous reports indicate that when planarians are chronically exposed to μM concentrations of cocaine, they display stereotypical withdrawal-like behaviors when the cocaine is removed. Here we report that parthenolide prevents this cocaine-induced expression of planarian withdrawal-like behaviors.
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Keywords: Planaria; Cocaine; Parthenolide; Sesquiterpene lactones; Withdrawal

The flatworm *planaria* is a useful animal model to study cocaine effects in biological systems. This organism has a simple nervous system, including a rudimentary brain, with every major neurotransmitter system described in mammals, including humans (Sarnat and Netsky, 1985). Additionally, its nerve cells share many structural similarities with vertebrate neurons (Sarnat and Netsky, 1985). Several previous studies examining the behavioral and morphological effects of cocaine on planaria have been published (Palladini et al., 1996; Margotta et al., 1997; Raffa and Valdez, 2001; Raffa and Desai, 2005).

Parthenolide is a naturally-occurring sesquiterpene lactone usually isolated from the feverfew plant (*Tanacetum parthenium)*, which is used as an anti-migraine agent (Tassorelli et al., 2005). We recently reported that parthenolide and related compounds were able to prevent and reverse behavioral responses in planarian worms in an acute cocaine exposure model (Pagán et al., in press). Here we report that parthenolide prevents the expression of withdrawal-like behaviors in planarians in a model of chronic cocaine exposure.

Planarian worms (*Dugesia dorotocephala*) were purchased from Ward's (Rochester, NY). General laboratory materials and supplies were purchased from Fisher Scientific (Suwanee, GA). Parthenolide and (−)Cocaine hydrochloride were purchased from Tocris (Ellisville, MO) and from Sigma-Aldrich (St. Louis, MO), respectively. All the experiments described here were done at room temperature using artificial pond water (APW, NaCl, 6 mM; $NaHCO_3$, 0.1 mM; $CaCl_2$, 0.6 mM; pH 6.9), containing 0.1% dimethylsulfoxide (DMSO) as a solubility-aiding agent. At this concentration, DMSO does not have any apparent behavioral or toxic effects in planaria (Pagán et al., 2006). Planarians were transferred to APW upon arrival and left to get adjusted to the laboratory conditions for at least 24 h before the experiments.

The worms were used within two weeks of arrival, and the APW was changed daily. All graphs and statistical procedures were done using the GraphPad 5/Instat software packages (GraphPad Software, San Diego, CA).

The procedure we used was adapted from a published method (Raffa and Desai, 2005). Briefly, planarians were placed into separate 1.5 ml microcentrifuge tubes containing 100 μM cocaine, 5 μM parthenolide or the combination of the two. Two sets of control worms were also observed, planarians pre-exposed to plain APW or to APW/0.1% DMSO. After an overnight incubation period (22–27 h), the worms were individually transferred to glass

* Corresponding author. Department of Biology, West Chester University, 750 S. Church St., West Chester, PA 19383-2112, United States. Tel.: +1 610 436 2165; fax: +1 610 436 2183.
E-mail address: opagan@wcupa.edu (O.R. Pagán).

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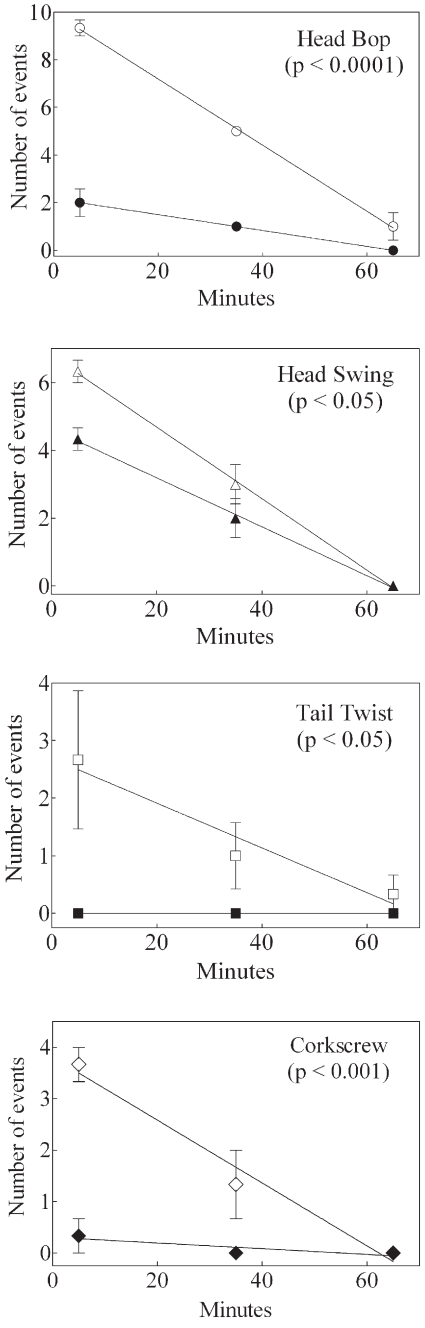

Fig. 1. Parthenolide significantly decreases the expression of cocaine-induced withdrawal-like behaviors in planaria. The specific behaviors are indicated in the plots. The open and closed symbols represent planarians exposed to 100 μM cocaine in the absence and in the presence of 5 μM parthenolide, respectively. The lines were generated by fitting the data to a linear equation. In each case, the lines in the presence and in the absence of parthenolide were compared to each other using the *F*-test. The *p*-values associated with the statistical test are shown in the plots. Each line includes the average of 3–5 worms. Error bars represent the standard error of the mean.

dishes containing APW in the absence of any experimental compounds and observed with a dissecting microscope during three time periods: 0–5, 30–35 and 60–65 min. The withdrawal-like behaviors observed were: "HeadBop" ("nodding"-like movement of head while gliding at the bottom of the dish), "HeadSwing" (rotation of the head in the absence of gliding while the tail is fixed to the bottom of the dish), TailTwist" (bending of the tail tip) and "Corkscrew" (spiral rotation while swimming). In contrast to the original paper (Raffa and Desai, 2005), we observed that two other described movements: "Squirming" (shaking) and "Clinging" (scrunching) tended to appear concurrently, therefore, we decided to count these movements together. The data was graphed as the number of events as a function of time (Fig. 1) and fit to a linear equation. The slopes (100 μM cocaine *vs.* 100 μM cocaine +5 μM parthenolide) were compared as described in the figure legend.

None of the control worms, nor the worms pre-exposed to 0.1% DMSO or 5 μM parthenolide alone displayed stereotypical behaviors upon transfer to plain APW (data not shown). In contrast, planarians pre-exposed to 100 μM cocaine as described above, displayed withdrawal-like behaviors when transferred to plain APW. As previously reported (Raffa and Desai, 2005), the number of events decreased as a function of time (Fig. 1, open symbols). Interestingly, preincubation with 100 μM cocaine plus 5 μM parthenolide, significantly reduced the counts for "Head-Bop", "HeadSwing", "TailTwist" and "Corkscrew" upon transfer to plain APW (Fig. 1 closed symbols). The worms also displayed squirming/clinging behaviors induced by cocaine which decreased over time, but these were not prevented by parthenolide (data not shown).

We have previously demonstrated that parthenolide and cocaine do not interact directly with each other (Pagán et al., in press). The simplest explanation for our results is that the putative binding sites for cocaine and parthenolide in planarian worms are related. The characterization of the [^{3}H]-cocaine binding sites in planaria is currently in progress in our laboratory. These experiments will most likely provide information about the relationship between these binding sites.

In summary, we demonstrated that parthenolide may provide innovative directions in the search for cocaine antagonists. Future studies will include additional cyclic sesquiterpene lactones examples in order to elucidate structure–function relationships.

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References

Margotta, V., Caronti, B., Meco, G., Merante, A., Ruggieri, S., Venturini, G., Palladini, G., 1997. Effects of cocaine treatment on the nervous system of planaria (*Dugesia gonocephala* s.l.). Histochemical and ultrastructural observations. Eur. J. Histochem. 41, 223–230.

Palladini, G., Ruggeri, S., Stocchi, F., De Pandis, M.F., Venturini, G., Margotta, V., 1996. A pharmacological study of cocaine activity in planaria. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 115, 41–45.

Pagán, O.R., Rowlands, A.L., Urban, K.R., 2006. Toxicity and behavioral effects of dimethylsulfoxide in planaria. Neurosci. Lett. 407, 274–278.

Pagán, O.R., Rowlands, A.L., Azam, M., Urban, K.R., Bidja, A.H., Roy, D.M., Feeney, R.B., Afshari, L.K., in press. Reversal of cocaine-induced planarian behavior by parthenolide and related sesquiterpene lactones. Pharmacol. Biochem. Behav. [Dec 15; Electronic publication ahead of print].

Raffa, R.B., Valdez, J.M., 2001. Cocaine withdrawal in planaria. Eur. J. Pharmacol. 430, 143–145.

Raffa, R.B., Desai, P., 2005. Description and quantification of cocaine withdrawal signs in planaria. Brain Res. 1032, 200–202.

Sarnat, H.B., Netsky, M.G., 1985. The brain of the planarian as the ancestor of the human brain. Can. J. Neurol. Sci. 12, 296–302.

Tassorelli, C., Greco, R., Morazzoni, P., Riva, A., Sandrini, G., Nappi, G., 2005. Parthenolide is the component of *Tanacetum parthenium* that inhibits nitroglycerin-induced Fos activation: studies in an animal model of migraine. Cephalalgia. 25, 612–621.