Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 9645–9648, October 1993
Neurobiology

Cocaine as a naturally occurring insecticide

(monoamines/octopamine/plant defense/antidepressants/transporters)

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Communicated by Melvin J. Cohen, June 10, 1993

ABSTRACT **Although cocaine has a fascinating and complex medicinal history in man, its natural function in plants is unknown. The present studies demonstrate that cocaine exerts insecticidal effects at concentrations which occur naturally in coca leaves. Unlike its known action on dopamine reuptake in mammals, cocaine's pesticidal effects are shown to result from a potentiation of insect octopaminergic neurotransmission. Amine-reuptake blockers of other structural classes also exert pesticidal activity with a rank order of potency distinct from that known to affect vertebrate amine transporters. These findings suggest that cocaine functions in plants as a natural insecticide and that octopamine transporters may be useful sites for targeting pesticides with selectivity toward invertebrates.**

Cocaine is obtained from the leaves of coca plants (*Erythroxylum* spp.). The four major varieties of coca that produce cocaine contain levels ranging from 0.35–0.72% dry weight, sometimes exceeding 1% in newly emerging leaves (1). Although relatively little is known about the natural insect pests of coca, Plowman and Weil (2) have observed that "compared with other tropical American crops, *E. coca* and *E. novogranatense* are relatively pest-free. Herbivorous insects are only rarely observed on the plants in the field; damage to leaves is often minor. This is especially noteworthy since, during much of the year, the membranaceous leaves of coca are found in the tender state of unfolding, the result of their being stripped 3–6 times a year during harvest". Because of this observation and because of cocaine's known anorexic effects in mammals (3, 4), it was of interest to examine cocaine's effect on feeding in insects.

METHODS

To measure the ability of cocaine and other drugs to protect leaves from insect feeding, a group of five 3-day-old *Manduca sexta* larvae (hatched on artificial medium) were placed on isolated and continuously hydrated tomato leaves which had been presprayed (0.75 μl of spray per mg of leaf) with a fine aerosol of drug or vehicle and allowed to dry (see refs. 5 and 6 for details). Drugs were dissolved in methanol, which, by itself, had no effect on feeding. The amount of leaf remaining was measured at 12- to 24-hr intervals and at the end of 72 hr of feeding. In each experiment, a dose–response curve was run. At each dose, drugs were tested in duplicate and each area measurement, at each time period, was done twice, blind, by two observers. All drugs were retested in separate experiments from two to six times. Relative leaf-protecting potencies of different drugs (shown in text) were based upon the mean IC_{50} values for a given drug. All drugs shown in Figs. 1 and 2 are known to be stable at room temperature.

The ability of cocaine to block Na^+-dependent amine uptake was measured essentially by the method of Evans (7), utilizing intact, hemisected *Blaberus* brains that were preincubated in Grace's insect medium (GIBCO) and then incubated at 25°C in insect saline (7) for 10 min with 1 μM [^{3}H]dopamine (DuPont) or 1 μM (−)-[^{3}H]octopamine in the presence or absence of various concentrations of (−)-cocaine hydrochloride (Research Biochemicals). Brains were then washed twice (1 min) in 50 ml of ice-cold saline lacking tracer, tissue was solubilized, radioactivity was quantitated, and uptake was compared with that of controls incubated in Na^+-free Tris-substituted saline. Na^+-dependent uptake was also measured, by the technique of ref. 8, in synaptosomal preparations from *Blaberus* thoracic ganglia and brain, using minor modifications necessary because of differences in density between insect and mammalian nerve tissue. Presence of intact synaptosomes was verified by electron microscopy. Results with the two types of tissue preparations were similar. [^{3}H]Octopamine (34 Ci/mmol; 1 Ci = 37 GBq) was prepared from 3,5-dibromooctopamine, reduced with $^3H_2$, isolated by HPLC, and repurified periodically by thin-layer chromatography.

Methods for measurement of light emission from firefly lanterns (see Fig. 4) were as described (6, 9).

Reagents were obtained from Sigma. Cocaine derivatives and reuptake blockers were obtained from Sigma and from Research Biochemicals.

RESULTS AND DISCUSSION

Leaf Protection by Cocaine. To investigate cocaine's potential leaf-protecting effects, we used, as a model, first-instar *M. sexta* larvae placed upon tomato leaves presprayed with various concentrations of (−)-cocaine hydrochloride. After a few minutes of exposure to cocaine-sprayed leaves, larvae displayed marked behavioral abnormalities, including rearing, tremors, and walk-off activity. These behaviors increased in intensity as larvae began to feed and, at higher concentrations of cocaine, the larvae stopped feeding and died after 24–48 hr. As a result, leaves were protected (Fig. 1). Experiments using procaine and lidocaine (Fig. 1) showed that leaf protection was not due to the local anesthetic effects of cocaine, an observation consistent, also, with the fact that larvae exposed to cocaine initially demonstrated hyperactivity rather than hypoactivity. Cocaine was also found to disrupt hatching of larvae if *Manduca* eggs were briefly dipped in an aqueous solution (EC_{50} = 0.2%). In other experiments, cocaine killed mosquito larvae with an EC_{50} of about 0.01%.

To determine whether the leaf-protecting and toxic effects of cocaine observed *in vitro* might be relevant to the amounts of cocaine found naturally in coca plants, we calculated the

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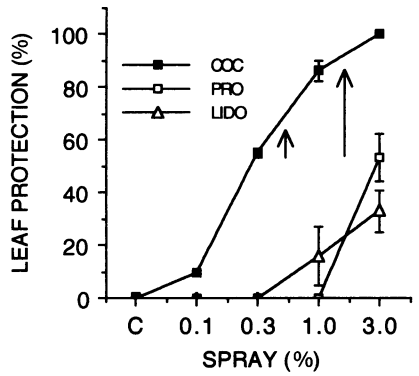

FIG. 1. Comparative effects of cocaine (■), procaine (□), and lidocaine (△) on protecting tomato leaves from being eaten by tobacco hornworms (*M. sexta*). For each drug concentration (%, wt/vol), quadruplicate measurements were made on two separate leaves (see *Methods*). Control (C), vehicle alone. Data shown for cocaine represent mean ± SEM for four separate experiments. Arrows show cocaine spray concentrations which replicate range of cocaine concentrations found naturally in coca leaves. Above a cocaine spray concentration of 0.5%, all larvae eventually died.

concentration of cocaine in fresh coca leaves by using data on alkaloid levels in dry leaves (1) and experimentally determining the water content (46–52%) in fresh specimens of six species of *Erythroxylum* (K. Pierce-Nathanson and J.A.N., unpublished data). Fig. 1 (arrows) shows that the amount of cocaine found in fresh coca leaves would result in 68–88% inhibition of *Manduca* feeding if the alkaloid were present in tomato leaves. For the higher cocaine content found in newly opened coca leaves, this figure would exceed 95% inhibition of feeding. Thus, at the concentrations found naturally in coca, cocaine protects leaves and is pesticidal.

Amine Transporter Antagonists. In mammals, low doses of cocaine cause stimulation and euphoria, while high doses cause anorexia, hyperactivity, tremors, incoordination, vomiting, and tonic–clonic convulsions (3, 4). Although recent literature has emphasized cocaine's action in blocking dopamine reuptake into mammalian presynaptic nerve terminals (thereby augmenting and prolonging dopaminergic neurotransmission), cocaine is also effective in blocking reuptake of norepinephrine and serotonin (3, 4, 10, 11). Thus, in insects, cocaine's antifeeding effects might result from the inhibition of reuptake of dopamine or some other amine.

To investigate this possibility, we examined the insect antifeeding effects of other amine-reuptake blockers known to have different degrees of selectivity toward dopamine, norepinephrine, and serotonin. Fig. 2*A* shows that several compounds were effective in protecting leaves, including desmethylimipramine (DMI) (a better blocker of norepinephrine reuptake than of serotonin or dopamine reuptake), amitriptyline (AMT) (a better serotonin than dopamine blocker), xylamine (XYL) (a selective blocker of norepinephrine reuptake), fluoxetine (FLU) (primarily a serotonin blocker), GBR-12909 (a relatively selective dopamine-reuptake blocker, and DSP-4 (an irreversible blocker of norepinephrine reuptake. Mazindol (MAZ), a blocker of dopamine and norepinephrine uptake, had little effect.

The rank order of potency of the various compounds for protecting leaves from insect attack (GBR > cocaine > DMI = AMT = XYL > FLU ≫ MAZ) differed substantially from their order of potency in mammalian cells for blocking uptake of dopamine (GBR ≫ MAZ ≫ cocaine > DMI = AMT = FLU > XYL) (12–19) or that of norepinephrine (MAZ > DMI > AMT = XYL > FLU > cocaine > GBR) (14, 15, 19–23) or serotonin (FLU > AMT > cocaine = DMI = MAZ > GBR > XYL) (18, 19, 21–24). Furthermore, when structural analogs of cocaine were evaluated for insect toxicity (Fig. 2*B*),

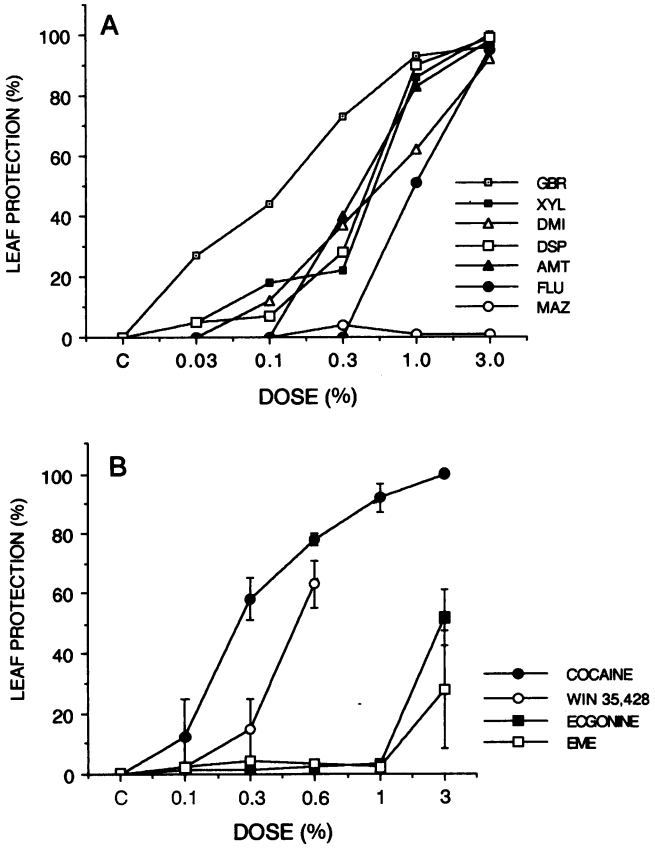

FIG. 2. (*A*) Leaf-protecting effect of amine-reuptake blockers with different degrees of selectivity for inhibiting dopamine, norepinephrine, or serotonin synaptosomal transport in mammalian tissues. (*B*) Comparative effects of cocaine analogs on protecting leaves. Procedures were as in Fig. 1. All drugs were evaluated in at least two separate experiments, and values shown are from a given representative experiment. GBR, GBR-12909; XYL, xylamine; DMI, desmethylimipramine; DSP, DSP-4 (an irreversible blocker or norepinephrine reuptake); AMT, amitriptyline; FLU, fluoxetine; MAZ, mazindol; EME, ecgonine methyl ester.

the relative rank order of potency observed [cocaine > WIN 35,428 ≫ ecgonine = ecgonine methyl ester (EME)] differed from the known rank order of potency of these analogs in blocking reuptake into mammalian brain of dopamine (WIN > cocaine > EME ≫ ecgonine), norepinephrine (WIN = cocaine ≫ EME = ecgonine), or serotonin (WIN > cocaine ≫ EME = ecgonine) (12, 18, 19). These pharmacological data suggested that cocaine and the other transporter blockers might be exerting their leaf-protecting effects in insects by affecting the uptake of some amine(s) other than dopamine, norepinephrine, or serotonin.

Relevant to this possibility, we noted that the behavioral effects of insects exposed to these compounds were quite similar to the adverse behavioral effects that occur when insects ingest agonists of the neurotransmitter and hormone octopamine, a norepinephrine-like amine found primarily in invertebrates and known to regulate insect motor, behavioral, and metabolic functions (5, 25, 26). The octopamine-like toxic effects of cocaine and the other active compounds suggested that these agents might be acting biochemically through a blockade of octopamine reuptake in insects, thereby augmenting octopamine neurotransmission and functionally acting as octopamine agonists.

High-Affinity Octopamine Uptake. Because a high-affinity octopamine-reuptake mechanism is known to exist in insects (7, 30, 31), we carried out biochemical studies with insect brain and ganglia and determined that cocaine was as effec-

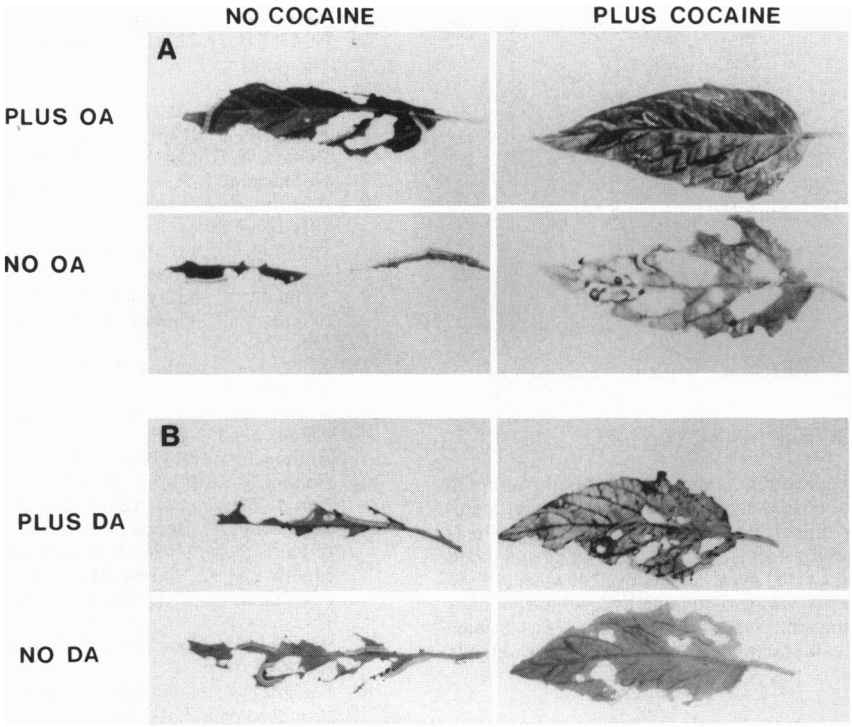

FIG. 3. Action of cocaine to augment the leaf-protecting effect of octopamine but not dopamine. (*A*) Condition of typical leaves 72 hr after hornworm larvae were placed on presprayed plants. A 1% (wt/vol) spray concentration of octopamine (OA), which was partially effective alone (*Upper Left*), when combined with a partially effective 0.6% concentration of cocaine (*Lower Right*), resulted in complete leaf protection (*Upper Right*). Control (solvent alone) is shown in *Lower Left*. (*B*) Comparable experiments with dopamine (DA) showed no leaf protection by dopamine and no enhancement by cocaine, indicating that the leaf-protecting effects of cocaine do not appear to involve dopamine, although cocaine can block the uptake of both octopamine and dopamine (see text).

tive in inhibiting the uptake of radioactive octopamine (IC_{50} = 115 ± 25 μM) as it was in blocking the uptake of radioactive dopamine (IC_{50} = 105 ± 25 μM) [mean ± SD or ± SEM of two (octopamine) or three (dopamine) independent experiments, each determining cocaine inhibition in duplicate; see *Methods*]. Because the concentration of cocaine found in fresh coca leaves (3–10 mM) considerably exceeds these IC_{50} values, larvae feeding to even a small extent on coca leaves would be expected to develop substantial inhibition of octopamine uptake. [Note that prior studies have shown that dopamine and octopamine can each compete with the other's reuptake site (7, 27, 28); therefore, the observed uptake of a particular amine may represent transport by both dopamine and octopamine transporters. Accordingly, relative physiological activity of antagonists (Fig. 2), as well as additivity studies (below), provided the strongest evidence that the toxic effects of cocaine are due to its effect on octopamine rather than dopamine.]

Potentiation of Octopamine Neurotransmission. Physiological evidence that the toxic effects of cocaine in insects is due to its inhibition of octopamine, rather than dopamine, uptake was obtained from additivity studies, in which it was predicted that cocaine should selectively augment the leaf-protective effects of octopamine, but not those of dopamine. Fig. 3 shows that this is what we observed; a concentration of octopamine which by itself was only partially effective in protecting leaves, when added to a partially effective concentration of cocaine, resulted in complete leaf protection. In contrast, dopamine alone (at doses up to twice that of octopamine) exerted no leaf protection, and addition of dopamine to cocaine caused no further increase in cocaine's effect. Likewise, at similar concentrations, norepinephrine had no leaf-protecting effect (data not shown).

Additional evidence demonstrating cocaine's ability to potentiate octopamine neurotransmission in insects was obtained by using the firefly neurogenic light response. Considerable biochemical and physiological evidence indicates that initiation of light emission in fireflies is mediated solely by octopamine-containing neurons entering the light organ (29). In this tissue, there is no evidence for receptors or for neurotransmission by dopamine, norepinephrine, or serotonin (6, 9). Fig. 4 shows that injection of a fixed dose of cocaine significantly potentiated the light-stimulating action of simultaneously injected octopamine, causing a leftward shift in the octopamine dose–response curve by a factor of about 10. (Cocaine itself had no activity as a direct octopamine receptor agonist.) These results indicate that cocaine can potentiate the action of octopamine and further suggest that compounds which act both as octopamine-reuptake blockers and as octopamine receptor agonists may have increased potency for disrupting insect behavior.§

Taken together, the data provide strong evidence that enhancement of octopaminergic neurotransmission is a significant factor in cocaine's toxicity in insects. Although care must be used when speculating about the role of secondary products in plants, it is possible that cocaine, like certain other alkaloids found in plants (e.g., nicotine, pyrethrum, caffeine), functions naturally as an insecticide in *Erythroxylum*. Whether cocaine's added ability to block amine reuptake in vertebrates was an evolutionary adaptation that conferred additional protection from mammalian predators is possible but seems unlikely given the strongly reinforcing

§The present results may also explain the otherwise paradoxical effect of an octopamine receptor antagonist, cyproheptadine, to augment rather than block the leaf-protecting effects of OA (J.A.N. and E.G.H., unpublished observations). This tricyclic compound is also an effective blocker of octopamine uptake (7), a characteristic which would act to potentiate octopamine's leaf-protecting action and offset cyproheptadine's better known action as a receptor antagonist (6, 9).

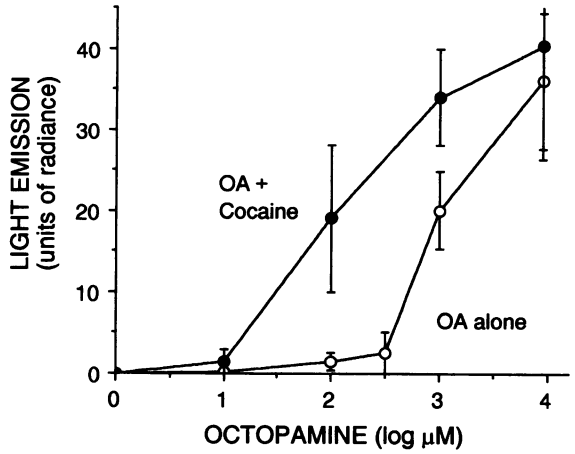

FIG. 4. Ability of cocaine to potentiate action of octopamine in eliciting light emission in the isolated firefly tail, a purely octopaminergic system that lacks innervation by other amines. Shown is maximal illumination resulting from injection of 3 μl of indicated concentration of octopamine (OA) in the absence (○) or presence (●) of simultaneously injected 500 μM (−)-cocaine hydrochloride. (Actual concentrations reaching the synaptic area were less.) Values shown are mean ± mean deviation (or ± SEM) of two to five animals per concentration.

effect of cocaine ingestion in mammals (which might encourage additional feeding on coca leaves). It is rather more likely that cocaine's use (and misuse) in vertebrates is an unrelated side effect of this compound's low degree of selectivity for blocking uptake of various amines. By the same token, because there is relatively little evidence for a normal role of octopamine in mammals, the present results suggest that new compounds with an ability to more specifically block octopamine reuptake may have potential as selective pesticides for insects with reduced toxicity and reduced potential for abuse by vertebrates.

We thank M. McKee and M. J. Deak for technical assistance, J. Hyde of Gateways Farms for tomato plants, and M. Collins of Fairchild Tropical Gardens, Miami, for specimens of *Erythroxylum*.

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