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Inhibition by cocaine of inositol phospholipid hydrolysis induced by the sodium channel activator batrachotoxin in mouse cerebral cortex

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Hydrolysis of inositol phospholipids has received much recent attention as an effector system coupled to α_1-adrenergic, 5-HT_2-, muscarinic-cholinergic, and certain peptidergic receptors, with diacylglycerol and inositol 1,4,5-trisphosphate as the second messengers [1, 2]. In addition to agonists acting on these receptors, various agents that activate voltage-dependent sodium channels have been found to stimulate polyphosphoinositide turnover in guinea pig synaptoneurosome preparations [3]. Thus, sodium channel activity *per se* can have an input into phosphatidylinositol systems. Cocaine is a potent blocker of neuronal monoamine uptake [4, 5]; the resulting increase in monoamine in the synaptic cleft possibly leads to an enhanced polyphosphoinositide turnover in neurons innervated by norepinephrine or serotonin. In addition, cocaine, at concentrations theoretically present in brains of cocaine addicts (for citations, see Ref. 6), is also a potent local anesthetic drug capable of blocking the sodium channel *in vitro* [7]. In contrast to the former effect, the inhibition of sodium flux by cocaine may lead to reductions in inositol phospholipid hydrolysis. The experiments described in this paper address this possibility. For comparison, the study also included the local anesthetic drug tetracaine, which has a relatively low affinity for carriers involved in monoamine uptake as measured by cocaine binding [8] and by inhibition of uptake of serotonin into plasma membrane vesicles derived from brain synaptosomes, indicating IC_{50} values of cocaine and tetracaine of 0.2 and 23 μM respectively (data not shown).

Materials and Methods

Batrachotoxin (BTX)* was a donation from Dr. J. W. Daly, NIH, Bethesda, MD. Cocaine hydrochloride was from Mallinckrodt, St. Louis, MO. Sigma (St. Louis, MO) was the supplier of tetracaine, tetrodotoxin (TTX), scorpion venom (*Leiurus quinquestriatus*) (ScVenom), and aconitine. The New England Nuclear Corporation (Boston, MA) supplied [^{3}H]inositol (54.5 Ci/mmol).

For all our experiments we used male BALB/cBy mice, 8–10 weeks of age, weighing 20–22 g, from the breeding colony of our Institute. Freshly dissected cerebrocortical tissue was cross-chopped (350 μm) and dispersed in 10 vol. of warm Krebs–Henseleit buffer gassed with O_2–CO_2 (95:5), pH 7.4 (for composition, see Ref. 9). [^{3}H]Inositol was added at a final concentration of 10 μCi/ml (approximately 1 μM), and the slices were incubated for 90 min at 37° under continuous gassing. Subsequently, the medium was removed, and the slices were rinsed with an excess of warm buffer. An appropriate amount of buffer containing 10 mM LiCl was added to the washed slices for a final concentration of approximately 0.4 g of tissue per ml of buffer. Aliquots of 70 μl were transferred to polypropylene tubes, 250 μl of buffer containing 10 mM LiCl was added, and the mixtures were incubated for 10 min at room temperature. Subsequently, drugs were added (20 μl) from stock solutions, and the tubes were briefly gassed and capped. After incubation for 60 min at 37°, the reaction was stopped by addition of 0.94 ml of methanol–chloroform (2:1). Accumulation of [^{3}H]inositol phosphates was measured as described by Berridge *et al.* [1]. The samples with methanol–chloroform were incubated for 10 min at room temperature. Subsequently, 310 μl of chloroform and 310 μl of water were added, and the tubes were shaken for 30 sec. After separation of the layers by centrifugation, 0.8 ml of the aqueous upper layer was placed on an AG 1-X8 anion exchange column (formate form, 0.5 ml). Free [^{3}H]inositol was eluted with three 4-ml portions of water, and [^{3}H]inositol phosphates with two 2-ml portions of 1 M ammonium formate/0.1 M formic acid. An aliquot of 0.4 ml was taken from the organic lower phase in the centrifuged tubes and evaporated to dryness overnight to estimate the incorporation of [^{3}H]inositol into the total lipid soluble mixture of phospholipids. The radioactivity in lipid soluble phospholipids was used as a measure of the amount of tissue in each incubation. The release of [^{3}H]inositol phosphates was calculated as percent of total tritium radioactivity incorporated into phospholipid. For each condition, the results were expressed as the percent of basal values obtained with buffer alone in the same experiment. Under the present conditions with lithium, the major product from the breakdown of phosphatidylinositol can be expected to be inositol phosphate [1]. In this study, the total accumulation of [^{3}H]inositol phosphate was used as a measure of phosphatidylinositol hydrolysis. Differences were statistically evaluated with the two-tailed Student's *t*-test.

The measurement of veratridine-stimulated [^{14}C]guanidine uptake was carried out by a combination of the protocols described by Tamkun and Catterall [10] and Pauwels *et al.* [11]. Purified synaptosomal preparations prepared from mouse cerebral cortex as described by us previously [6] were thawed and incubated for 30 min at 36° with 60 μM veratridine or various concentrations of cocaine and tetracaine in a total volume of 100 μl of preincubation buffer (for composition, see Ref. 10). The uptake assay was started by addition of 0.3 ml of uptake solution [10] containing the respective concentration of cocaine or tetracaine, 60 μM veratridine, 4 mM guanidine hydrochloride, and a trace amount of [^{14}C]guanidine hydrochloride (48 mCi/mmol) (Amersham Corp., Arlington Heights, IL). After 40 sec, 4 ml of ice-cold wash-solution [10] was added to stop the uptake, and the mixture was filtered through a prewetted Millipore filter (DAWP 0.65 μm). The filter was washed with 4 ml of wash-solution and assayed for radioactivity. Nonspecific guanidine uptake was determined with 1 μM tetrodotoxin in both the preincubation and uptake solutions, and amounted to 32% of the total uptake.

Results and discussion

Phosphoinositide hydrolysis in mouse cerebrocortical slices was stimulated by the sodium channel activators BTX and aconitine (Fig. 1), as demonstrated also by Gusovsky *et al.* [3] for synaptoneurosome preparations from guinea pig cerebral cortex. BTX stimulated inositide hydrolysis with an EC_{50} of approximately 20 nM (Fig. 2). This is lower than the EC_{50} of 500 nM reported for stimulation of sodium uptake by synaptosomes [10]. In contrast, the EC_{50} of 10 μM for aconitine in stimulating inositide hydrolysis (data not shown) is similar to the 14 μM EC_{50} value for the stimulation of synaptosomal sodium uptake [10]. In comparison with the synaptosome, the slice is a more complex system with

* Abbreviations: BTX, batrachotoxin; BTX-B, batrachotoxinin A 20-α-benzoate; ScVenom, Scorpion venom (*Leiurus quinquestriatus*); and TTX, tetrodotoxin.

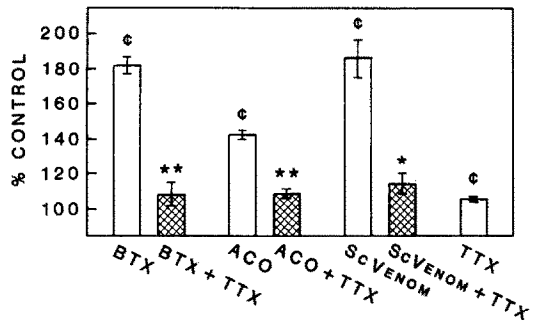

Fig. 1. Inhibition by 5 μM TTX of inositol phospholipid hydrolysis elicited by 1 μM BTX, 50 μM aconitine (ACO), or 5 μg/ml of scorpion venom (ScVenom). Slices labeled with [^{3}H]inositol were incubated with activator in the absence (open bars) or presence (hatched bars) of TTX for 60 min. Each value is the average of three to twelve separate measurements. Key: (¢) $P < 0.003$ compared with control values; (**) $P < 0.001$ compared with activator alone; and (*) $P < 0.01$ compared with activator alone. Inositide hydrolysis was measured by accumulation of inositol phosphate and expressed as percent of control. The incorporation of [^{3}H]inositol into phospholipids averaged 284,000 dpm/g of tissue (initial wet weight), and an amount of 44,800 dpm/g of tissue was released in the form of [^{3}H]inositol phosphates under control conditions.

many compartments and barriers, resulting in more complex, less direct relationships between the concentration of a drug in the medium and that at the receptor site. Maximal stimulation (200%) of phosphoinositide hydrolysis was found with a concentration of 100 nM BTX (Fig. 2). A lower maximal stimulation (145%) was found with concentrations of aconitine of $\geq$50 μM (Fig. 1) (data for higher concentrations not shown), consonant with other observations in sodium uptake assays, indicating that aconitine is a partial agonist and BTX a full agonist [10]. It is likely that the uptake of sodium underlies the observed stimulatory effects on inositide hydrolysis because ScVenom, which retards sodium channel inactivation, also increased the phosphatidylinositol response (Fig. 1). In addition, 5 μM TTX, a specific blocker of sodium channels, prevented the stimulation by 1 μM BTX, by 50 μM aconitine, and by 5 μg/ml of ScVenom (Fig. 1). It has been suggested that the stimulatory effect of sodium channel activators on inositide hydrolysis is due to a regulatory effect of intracellular sodium ions and not to the release of neurotransmitters caused by depolarization resulting from the sodium uptake by synaptoneurosome preparations; sodium could interact with guanine nucleotide regulatory proteins, or activate a sodium–calcium exchange [3]. A homeostatic cycle has been proposed consisting of activation of sodium channels, leading to formation of inositol trisphosphate and diacylglycerol; this, in turn, mobilizes Ca^{2+} and results in protein kinase C-catalyzed phosphorylation of sodium channels [3].

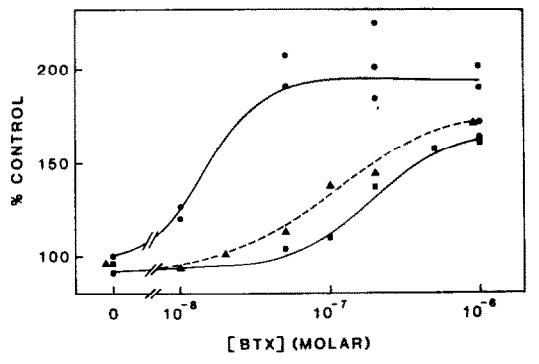

Fig. 2. Effects of cocaine and tetracaine on the activation of inositol phospholipid hydrolysis by BTX. Slices labeled with [^{3}H]inositol were incubated with various concentrations of BTX for 60 min in the absence (●—●) or presence of 300 μM cocaine (▲—▲) or 100 μM tetracaine (■—■). Each point is the average of three separate measurements. Inositide hydrolysis was measured by accumulation of inositol phosphate and expressed as percent of control. The incorporation of [^{3}H]inositol into phospholipids averaged 269,000 dpm/g of tissue (initial wet weight), and an amount of 49,300 dpm/g of tissue was released in the form of [^{3}H]inositol phosphates under control conditions.

The presence of 100 μM tetracaine increased the EC_{50} of BTX in stimulating inositide hydrolysis from approximately 20 nM to 0.2 μM (Fig. 2). A similar change was found with cocaine: the EC_{50} of BTX was approximately 0.1 μM in the presence of 300 μM cocaine. This, along with a reduction in the maximal stimulation, could indicate an uncompetitive mechanism of inhibition (Fig. 2). In BTX (0.05 μM)-activated slice preparations, tetracaine was quite potent in reducing the stimulated inositide hydrolysis (IC_{50} = 2 μM, Fig. 3A). Cocaine also counteracted the inositide hydrolysis stimulated by 0.05 μM BTX with an approximate IC_{50} value of 100 μM (Fig. 3A).

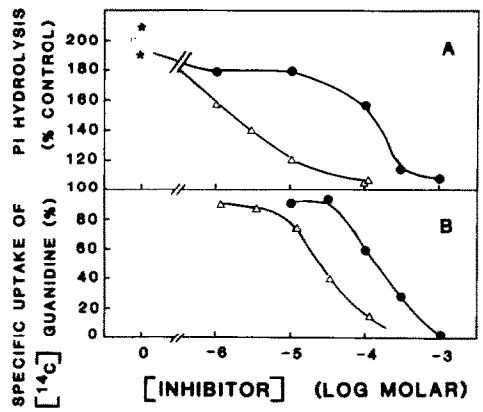

Fig. 3. Inhibition by cocaine and tetracaine of inositol phospholipid hydrolysis elicited by 0.05 μM BTX in slices and of [^{14}C]guanidine uptake into synaptosomes induced by 60 μM veratridine. Various concentrations of cocaine (●—●) or tetracaine (△—△) were present during incubation of slices, prelabeled with [^{3}H]inositol, with 0.05 μM BTX for 60 min (panel A) and during incubation of synaptosomes, activated with 60 μM veratridine, with [^{14}C]guanidine for 40 sec (panel B). Each point is the average of three determinations. Inositide hydrolysis was measured by accumulation of inositol phosphate and expressed as percent of control. Specific guanidine uptake into synaptosomes was defined as the difference between total uptake and uptake in the presence of 1 μM TTX. The incorporation of [^{3}H]inositol into phospholipids averaged 249,000 dpm/g of tissue (initial wet weight), and an amount of 40,300 dpm/g of tissue was released in the form of [^{3}H]inositol phosphates under control conditions. The specific guanidine uptake in the absence of drugs averaged 216 pmol/mg protein/sec. In panel A, the asterisks represent the values observed in the absence of inhibitors.

[^{14}C]Guanidine uptake into purified synaptosomes can be used as a model for measuring sodium flux as demonstrated by Pauwels *et al.* [11]. In the present study, veratridine (60 μM)-stimulated guanidine uptake into mouse cerebrocortical synaptosomes was measured over a time period of 40 sec during which the uptake was still linear with time (data not shown). Tetracaine and cocaine inhibited guanidine uptake with IC_{50} values of 25 and 140 μM respectively (Fig. 3B). Thus, tetracaine is more potent than cocaine in inhibiting both veratridine-stimulated guanidine flux in synaptosomes and BTX-stimulated inositide hydrolysis in slices, consonant with the idea that the two effects are related. Tetracaine has been shown to be more potent than cocaine also in other sodium channel assays: there is an 8-fold difference in potency between the drugs for inhibition of BTX-induced depolarization in synaptosomes [12], a 15-fold [12] or 16-fold [6] difference for inhibition of [^{3}H]BTX-B binding, and an 8-fold difference for acceleration of dissociation of [^{3}H]BTX-B binding from synaptosomes in the presence of aconitine [6]. In the inositide assay, tetracaine was more potent than cocaine as indicated by the shift to the right in the BTX activation curve in the presence of 100 μM tetracaine as compared with 300 μM cocaine (Fig. 2) and by the 50-fold difference in inhibiting potency after stimulation with 0.05 μM BTX (Fig. 3A). The high IC_{50} value of 100 μM for cocaine in inhibiting BTX (0.05 μM)-elicited inositide turnover (Fig. 3) makes the pharmacological relevance of this effect doubtful. However, the IC_{50} value will depend on the concentration of activator used, and it should be recalled that levels of cocaine in the brain of cocaine addicts can be expected to be as high as 2–10 μg/ml (7–33 μM) after a dose of cocaine normally administered intranasally, intravenously, or intrapulmonarily (for citations see Ref. 6). Such levels, if present at sodium channels, can be expected to inhibit sodium uptake and to affect the input of sodium channel activity into the phosphatidylinositol system. The present results do not negate the possibility that blockade of monoamine uptake by cocaine could lead to increased inositide hydrolysis in neurons receiving monoaminergic innervation. Perhaps a superfused and stimulated slice preparation could be used to demonstrate an inositol response as a result of monoamine uptake inhibition. The present results, however, do show that the increase in inositide hydrolysis induced by a sodium channel activator can be counteracted by concentrations of cocaine that inhibit the flux of guanidine through activated sodium channels. In addition, the correlation between the potencies of cocaine and tetracaine in inhibiting BTX-elicited inositide hydrolysis and those in inhibiting guanidine flux suggests that the former effect of cocaine is mediated by inhibition of sodium flux.

In conclusion, this study demonstrates that cocaine can interact with the phosphoinositide system at concentrations that inhibit sodium flux. The stimulation of inositide hydrolysis by BTX, aconitine, and ScVenom in mouse cerebrocortical slices was reduced by the presence of 5 μM TTX. Cocaine and tetracaine shifted the dose–response curves of BTX in stimulating inositide hydrolysis to the right. In addition, cocaine and tetracaine inhibited BTX (0.05 μM)-elicited inositide hydrolysis with IC_{50} values of approximately 100 and 2 μM respectively. Finally, cocaine and tetracaine inhibited veratridine (60 μM)-stimulated [^{14}C]guanidine uptake into synaptosomes with IC_{50} values of approximately 140 and 25 μM respectively.

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REFERENCES

1. M. J. Berridge, R. M. C. Dawson, C. P. Downes, J. P. Heslop and R. F. Irvine, *Biochem. J.* **212**, 473 (1983).
2. S. R. Nahorski, D. A. Kendall and I. Batty, *Biochem. Pharmac.* **35**, 2447 (1986).
3. F. Gusovsky, E. B. Hollingsworth and J. W. Daly, *Proc. natn. Acad. Sci. U.S.A.* **83**, 3003 (1986).
4. B. K. Koe, *J. Pharmac. exp. Ther.* **199**, 649 (1976).
5. M. E. A. Reith, B. E. Meisler, H. Sershen and A. Lajtha, *Biochem. Pharmac.* **35**, 1123 (1986).
6. M. E. A. Reith, S. S. Kim and A. Lajtha, *J. biol. Chem.* **261**, 7300 (1986).
7. S. W. Postma and W. A. Catterall, *Molec. Pharmac.* **25**, 219 (1984).
8. M. E. A. Reith, H. Sershen and A. Lajtha, *Life Sci.* **27**, 1055 (1980).
9. E. B. Hollingsworth, E. T. McNeal, J. L. Burton, R. J. Williams, J. W. Daly and C. R. Creveling, *J. Neurosci.* **5**, 2240 (1985).
10. M. M. Tamkun and W. A. Catterall, *Molec. Pharmac.* **19**, 78 (1981).
11. P. J. Pauwels, J. E. Leysen and P. M. Laduron, *Eur. J. Pharmac.* **124**, 291 (1986).
12. C. R. Creveling, E. T. McNeal, J. W. Daly and G. B. Brown, *Molec. Pharmac.* **23**, 350 (1983).

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Scavenging of hypochlorous acid by tetracycline, rifampicin and some other antibiotics: a possible antioxidant action of rifampicin and tetracycline?

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Antibiotics are widely used in the treatment and prophylaxis of acute and chronic infections. As well as their antibacterial actions, they have multiple effects on the immune system (reviewed in [1]). For example, several antibiotics have been reported to decrease bacterial killing by activated phagocytes, and the possibility that antibiotics might interfere with phagocyte killing mechanisms has been raised [1–3]. Activated phagocytes produce [4, 5] superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and, possibly, the highly-reactive hydroxyl radical (·OH), all of which are important agents in bacterial killing and in the damage to surrounding tissues that can be produced by activated phagocytic cells