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Biochemical Pharmacology, Vol. 29, pp. 1861–1863.

0006-2952/80/0615-1861 $02.00/0

Cocaine: effect of *in vivo* administration on synaptosomal uptake of norepinephrine

(*Received* 24 *September* 1979; *accepted* 11 *December* 1979)

Ever since reports of coca leaf chewing filtered back to Europe in the wake of the Spanish conquest of the Andes, interest in cocaine has waxed and waned in sympathy with social currents which encouraged or discouraged its use. Consequently, with the increase in illicit cocaine traffic which has taken place since the mid-1960's [1], research interest in cocaine has been rejuvenated. Recent investigations have confirmed that cocaine is a central nervous system stimulant. As currently reviewed [2], in humans cocaine significantly reduces total and rapid eye movement (REM) sleep. It produces euphoria, anorexia, perceptual and affective changes at low doses, and paranoid psychoses at higher doses. In experimental animals, cocaine increases locomotor activity and body temperature, and elicits stereotyped behavior and turning toward the lesioned side in animals with unilateral lesions of the nigro-striatal dopaminergic pathway. It also reduces food intake, induces a desynchronized electroencephalogram (EEG), and increases multiple unit activity in the reticular formation.

It has been theorized that the CNS excitation may result from inhibition of reuptake of endogenously produced catecholamines, particularly norepinephrine (NE) [3], though an effect of *in vivo* cocaine on catecholamine uptake in brain tissues has not been demonstrated previously. Reuptake is considered to be the primary mechanism by which catecholamines are inactivated [4]. Inhibition of this process would permit neurotransmitter to linger within the synaptic cleft and prolong any post-synaptic actions of released neurotransmitter. Reuptake is mediated by an energy dependent, high affinity transport system (uptake 1) located at the axonal membrane of neurons [5] and is highly sodium-ion dependent. In the peripheral nervous system, cocaine has been shown to be a potent inhibitor of uptake 1 but has little effect on uptake 2, a high capacity, low affinity system with many differing characteristics from uptake 1 [6, 7].

The action of cocaine is on the neuronal membrane, while it is less effective in inhibiting uptake by granular (vesicular) membrane [8]. Moreover, the uptake of NE by sympathetic nerve cell bodies appears to be considerably less sensitive to cocaine inhibition than the uptake of this transmitter by nerve endings [9, 10]. The inhibition is competitive [11, 12], that is, cocaine vies with NE for uptake receptor sites.

Though cocaine inhibition of NE uptake has been demonstrated both *in vitro* and *in vivo* in a large number of peripheral tissues [2], unfortunately less data are available for the central nervous system. Moreover, the results have been contradictory. Decreased NE uptake has been reported using *in vitro* incubations with mouse synaptosomes [13], brain slices of cats [14], mice [15] and rats [11], chopped rat brains [16] and rat pineals [17]. But the rare *in vivo* studies have not demonstrated decreased NE uptake, though a reduction in [^{3}H]NE retention after its intraventricular injection into cocaine-treated rats has been reported [18]. Intraventricular injections of tritiated NE showed no decrease in NE uptake in animals receiving 15 mg/kg of cocaine compared with controls [19], and brain slices of mice treated with 40 mg/kg of cocaine took up tritiated NE to the same extent as saline-treated animals [15]. This suggested that cocaine might not have the same action in the brain as it does in the peripheral nervous system. However, the numerous problems in methods, including their sensitivity, coupled with the uncertainties in the interpretation of the results of *in vivo* uptake studies carried out in intact brain preparations, as outlined by Maxwell *et al.* [12], mitigate against the use of these techniques in characterizing the CNS actions of cocaine. We chose to surmount the CNS *in vivo* problems by performing an *in vivo* cocaine study on nerve endings that would subsequently be isolated from supporting glia and the blood–brain and liquor–brain barriers (synaptosomes). This would permit us to see if they would react to endogenously bound cocaine as they do to the drug *in vitro*. The synaptosomal preparation also permitted us to examine such discrete details of NE uptake as K_m and V_{max} (Michaelis–Menten analysis).

Adult male Sprague–Dawley rats, each weighing 350 g.

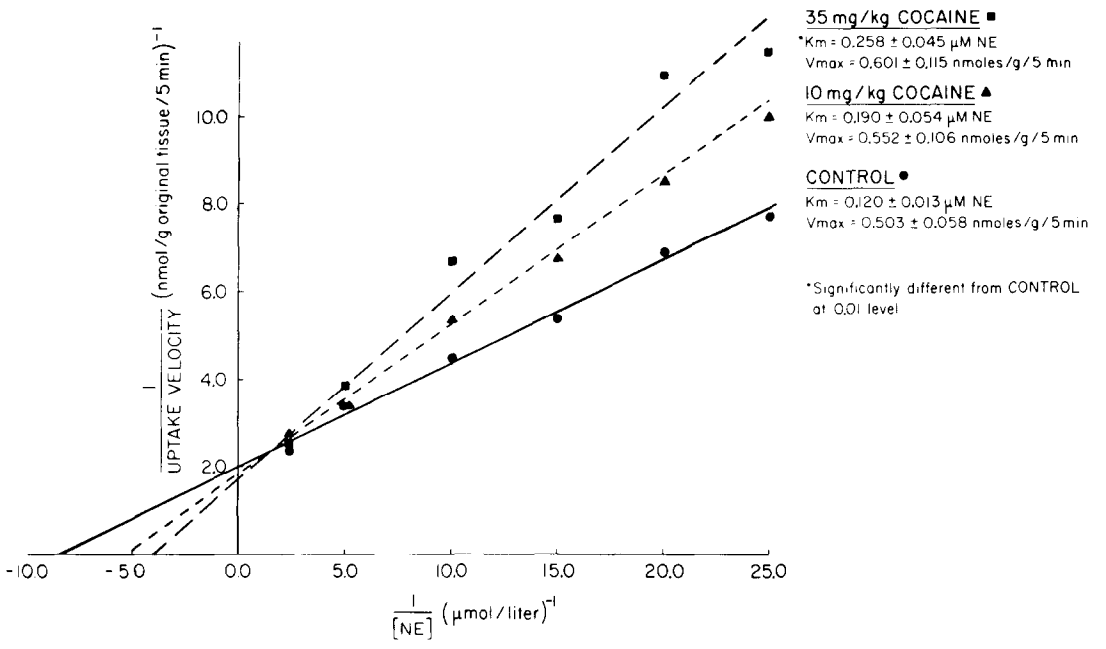

Fig. 1. Effect of cocaine on synaptosomal uptake of [^{3}H]NE. Double reciprocal plot of uptake velocity (in nmoles of *l*-norepinephrine taken up per gram of original tissue per 5 min) against the μM concentration of *l*-norepinephrine.

(Flow Laboratories, Dublin, VA), were injected i.p. with 1 cm^3 of either cocaine–HCl dissolved in physiological saline (10 mg/kg, N = 9, and 35 mg/kg, N = 9) or physiologic saline alone (N = 17). Thirty min later, they were decapitated, their brains were rapidly removed, and synaptosome-rich homogenates were prepared from the cortices. Dopamine (DA) cortical areas were dissected away beforehand so as not to interfere with NE uptake. The synaptosomes were suspended in enriched Krebs–Henseleit bicarbonate medium and were incubated with tritiated NE in six different concentrations ranging from 0.04 to 0.4 μM. For greater detail see the method of Hendley *et al.* [20]. Net active uptake velocities were calculated for each sample by correcting for blanks and non-specific uptake. These data were subjected to a least-squares non-linear analysis [21] to estimate K_m and V_{max} for each individual experiment and these were compared using Student's one-tailed *t*-test. The intercepts were plotted using the familiar double-reciprocal method (Fig. 1). Cocaine dose was plotted against K_m on semilog graph paper to obtain the ED$_{50}$ for NE uptake inhibition.

The results are what one would expect if the drug indeed acted *in vivo* as it has been shown to act *in vitro*. A typical pattern of competitive inhibition is illustrated (increase in K_m, see *x*-intercepts, and no change in V_{max}, see *y*-intercepts). Further, the amount of inhibition depended upon the dose of cocaine. Though only the higher drug dose created a significant increase in K_m ($P < 0.05$), the changes produced by the lower dose were in the predicted direction. Using semilog graph paper to plot dose vs K_m, the ED$_{50}$ (50 per cent inhibition of control NE uptake) for cocaine was 8 mg/kg. The increased K_m (decreased NE affinity) indicates that the injected cocaine interacted with NE uptake receptors in the pre-synaptic membrane of intact brain in a manner similar to that noted when cocaine is added to brain tissue in the test tube. The unaltered V_{max} indicates that the drug neither decreased the number of uptake receptors present nor their ability to transport neurotransmitter. In other words, there was no element of non-competitive inhibition.

The *in vivo* results shown here are an extension of previous findings. They permit us to conclude that competitive inhibition of NE uptake at nerve terminals does indeed comprise a major action of cocaine in the central nervous system. This uptake effect has been anticipated for some time but its demonstration has proved elusive. A complete picture of the effects of cocaine on NE uptake has been more difficult to develop in the CNS than in the periphery. In addition to the *in vivo* problems described earlier, the uptake changes in intact brain may be masked by effects of cocaine on synthesis, turnover, concentration, metabolism and release of NE. Because of the paucity of CNS cocaine studies and the conflicting data in the few studies thus far performed, details of these latter events for NE have not yet been sorted out. However, the discrepancies could also arise from the differences in experimental design. While some workers have reported no change in synthesis of telencephalic or hypothalmic NE after administration of cocaine and either intraventricular or intravenous injections of labeled tyrosine, an NE precursor [22–25], others have reported decreased NE synthesis after both tritiated tyrosine injections and administration of α-methyl paratyrosine, an NE synthesis inhibitor [23, 24]. A similar conflict (no change vs decrease) has resulted after attempts to measure endogenous NE concentrations following cocaine administration [25–27].

Since it is now possible to study concentration, synthesis, turnover and metabolism of neurotransmitters in synaptosomes, with almost the same ease as uptake and release, it may be possible to arrive more closely at the total orchestration of the effects of cocaine on central catecholamine functions, as well as those of other neurotransmitters, by studying these effects in isolated nerve endings themselves.

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Biochemical Pharmacology, Vol. 29, pp. 1863–1865.

0006-2952/80/0615–1863 $02.00/0

The influence of three prostaglandin biosynthesis stimulators on carrageenin-induced edema of rat paw

(*Received* 26 *July* 1979; *accepted* 3 *January* 1980)

Paracetamol [1] and four pyrazol–piridine derivatives [2] were found to stimulate prostaglandin biosynthesis. Phenol [3] and its iododerivative [4] possess the same property, which is supposed to be correlated with anti-inflammatory activity [3]. In contrast inhibition of prostaglandin biosynthesis has been accepted as the mode of action of aspirin-like anti-inflammatory drugs [5].

We compared the effect of 6-(beta-pyridyl)-3-hydroxy-pyrazol (3, 4b)-piridine (PPD) with that of 2-aminomethyl-4-*t*-butyl-6-iodophenol (MK-447) and paracetamol on cyclo-oxygenase and lipoxidase activity *in vitro* as well as on experimental inflammation *in vivo*.

The influence of tested compounds on cyclo-oxygenase activity was tested using solubilized enzyme from ram seminal vesicle microsomes. Microsomes were prepared according to the method of Smith [6], except that the concentration of diethyldithiocarbamate used was 5 mM. Then they were solubilized by the same medium as in the original paper [6]. The solubilized enzyme was diluted 15-fold with 0.1 M phosphate buffer, pH 7, and oxygen consumption and malondialdehyde generation was measured as previously described [2]. The results were expressed in μmoles of oxygen consumed by 1 mg of protein during 1 min. The initial reaction velocity was calculated from the slope of the line obtained on the oxygen monitor recorder. Malondialdehyde was estimated after 2 min incubation and its amount was expressed in nmoles produced during 1 min by 1 mg of protein. The protein was determined by the method of Lowry *et al.* [7].

Crystalline soybean lipoxidase was dissolved in 0.1 M phosphate buffer, pH 7, at a concentration of 5 μg/ml. Arachidonic acid (100 μM) was used as the substrate. Samples were incubated at 25°. The enzymic activity was measured as μmoles of oxygen consumed by 1 mg of enzyme during 1 min. Initial reaction velocity was calculated.

Carrageenin edema of hind paw in rats was produced by the method of Winter *et al.* [8]. Tested compounds were given s.c. 1 hr before the carrageenin injection. The increase in foot volume was expressed as a percentage of the volume before the carrageenin injection. In some experiments PPD was injected in a volume of 0.2 ml at a concentration of 5% and foot volume was measured every hour until the fourth hour. Wistar rats weighing 150–200 g were used.

The reagents used were lipoxidase from soybean (Sigma), carrageenin (Marine Colloids), paracetamol ("Polfa", Poland), diethydithiocarbamic acid (Sigma), flufenamic acid (Parke Davis), MK-447 (Merck, Sharp & Dohme). PPD was synthesized in The Department of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland.

The influence on cyclo-oxygenase activity in ram seminal