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Short communication

CENTRAL AND PERIPHERAL ADRENERGIC BLOCKING ACTIONS OF LSD AND BOL

Gene C. PALMER and Thomas F. BURKS *

Departments of Anatomy and Pharmacology, The University of New Mexico School of Medicine, Albuquerque, New Mexico 87106, USA

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Lysergic acid diethylamide (LSD) and its 2-bromo derivative BOL effectively antagonized the elevations in cyclic AMP produced by addition of norepinephrine to rat brain slices in vitro. LSD and BOL also exhibited antiadrenergic actions in dog isolated arteries (alpha receptors) and in rabbit isolated hearts (beta receptors).

Cyclic AMP
Central nervous system receptors
Norepinephrine
Lysergic acid diethylamide
Bromo-lysergic acid diethylamide
Antiadrenergic drugs

1. INTRODUCTION

Cyclic 3′,5′ adenosine monophosphate (cyclic AMP) has been shown to be a second messenger mediating the response of many hormones in several tissues (Robison et al., 1969). In the rat brain the catecholamines (norepinephrine, epinephrine, and isoproterenol) are the most potent known compounds capable of elevating cyclic AMP levels. The cyclic AMP response to catecholamines is blocked by classic alpha and beta adrenergic receptor blocking agents as well as by several phenothiazines and 5-hydroxytryptamine, which suggests that the rat brain adenyl cyclase receptor for adrenergic amines is relatively nonspecific (Palmer et al., 1971a,b). It has also been demonstrated that adrenergic blocking agents, chlorpromazine, and lysergic acid diethylamine (LSD) bind to nerve ending fractions which contain high adenyl cyclase activity (DeRobertis, 1971). The present study was performed to determine whether the hallucinogen LSD and its derivative, bromo-lysergic acid diethylamide (BOL), modify the elevation of cyclic AMP elicited by norepinephrine (NE) in slices of rat hypothalamus and brain stem. Cocaine, which inhibits adrenergic neuronal uptake of NE, was also tested. The ability of LSD and BOL to modify NE-induced vasoconstriction in isolated arteries and increases in the contractile force of isolated hearts was examined to help determine the receptor specificity of any adrenergic blockade produced by these compounds.

* Present address: Department of Pharmacology, The University of Texas Medical School at Houston, Houston, Texas 77025, USA.

2. METHODS

Young adult male rats (Sprague-Dawley, Holtzman, 140–160 g) were decapitated and the brains rapidly removed and placed in cold Krebs–Ringer bicarbonate buffer. Each hypothalamus and brain stem was divided into 3 portions, sliced (0.75 mm), and preincubated in Krebs–Ringer bicarbonate buffer for 30 min while 95% O_2–5% CO_2 was bubbled

into individual samples. Paired areas of the hypothalamus and brain stem were used in the cocaine experiments. The buffer was changed and either a control solution, LSD, BOL, or cocaine (each at 10^{-5} M) was added and the incubations were continued for 14 additional minutes at which time NE (5×10^{-5} M) was added. Six min later the samples were homogenized in 0.1 N HCl containing ^{3}H cyclic AMP. An aliquot was removed for protein determination and the homogenates were centrifuged, cyclic AMP isolated, determined, and expressed as picomoles of cyclic AMP formed per mg tissue protein as previously described (Butcher et al., 1965; Kakiuchi and Rall, 1968). All samples were assayed at two dilutions in triplicate using code numbers until the final data were calculated.

Contractile force was measured in spontaneously beating hearts isolated from albino rabbits and perfused through an aortic cannula with warm, aerated Krebs bicarbonate solution (Burks and Cooper, 1967). Perfusion pressure was measured in dog mesenteric arteries perfused in vitro at constant rates of flow with Krebs bicarbonate solution in a non-recirculating system (Rogers et al., 1966). In both isolated hearts and isolated arteries, dose–response curves to agonist drugs were obtained in the absence and in the presence of antagonist drugs. In separate experiments, the effects of varying concentrations of antagonists on responses to single dose levels of agonists were also investigated. Control responses were again determined after the antagonist solution was washed out. Each preparation served as its own control; statistical analyses were performed by Student's *t*-test, paired comparison (Goldstein, 1964).

3. RESULTS

The effects of LSD, BOL, and cocaine on the response of cyclic AMP to NE in the rat hypothalamus and brain stem are shown in table 1. Cocaine was without effect in this system while LSD and BOL effectively reduced the cyclic AMP response to NE in both tissues. LSD, BOL and cocaine did not alter basal levels of cyclic AMP.

The vasoconstrictor responses (increases in perfusion pressure) to 2 μg NE and to 10 mg KCl were tested in the presence of phentolamine, LSD, and BOL. The vasoconstrictor response to NE, but not to KCl, in isolated mesenteric arteries is an α-adrenergic response. Phentolamine, an established α-adrenergic receptor blocking agent, was included in the protocol as a standard agent. Responses to NE were diminished 34±9% by 1×10^{-7} M phentolamine ($p<0.05$) and 25±7% by 1×10^{-6} M LSD ($n=7$, $p<0.05$); responses to KCl were not reduced by either of these agents.

Table 1
Effects of LSD, BOL and cocaine on norepinephrine-induced increases in rat brain cyclic AMP levels.

	Cyclic AMP pmoles/mg tissue protein [a]	
	Hypothalamus	Brain stem
Series 1		
Control	58.6 ± 2.4 (3) [b]	46.8 ± 6.7 (3)
LSD alone	59.5 ± 9.9 (3)	33.7 ± 10.1 (3)
BOL alone	48.0 ± 12.4 (3)	31.3 ± 6.0 (3)
NE alone	226.2 ± 49.1 (5)	122.0 ± 9.3 (4)
LSD + NE [c]	77.5 ± 14.2 (5)	66.5 ± 5.4 (5)
BOL + NE [c]	54.2 ± 15.7 (5)	75.4 ± 11.6 (5)
Series 2		
Control	100.8 ± 16.1 (4)	84.5 ± 11.3 (4)
Cocaine alone	105.9 ± 13.8 (4)	88.6 ± 20.4 (4)
NE alone	308.9 ± 42.3 (5)	365.2 ± 52.3 (5)
Cocaine + NE [c]	278.9 ± 64.8 (5)	332.9 ± 22.7 (5)

a) Cyclic AMP levels in slices of rat hypothalamus and brain stem incubated without drugs (control), with LSD, BOL or cocaine at 1×10^{-5} M, or with norepinephrine (NE) at 5×10^{-5} M.
b) The values represent picomoles of cyclic AMP per mg of protein ± S.E.M. Numbers in parentheses refer to the number of experiments.
c) Incubated 14 min with the antagonist alone plus 6 min after NE was added.

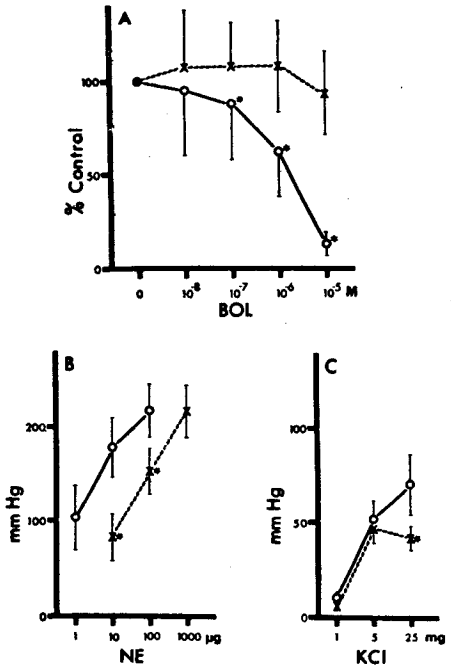

Fig. 1. Effects of bromo-lysergic acid diethylamide (BOL) on vasoconstrictor responses in dog isolated mesenteric arteries induced by norepinephrine (NE) and KCl. Each point represents mean (± S.E.) responses in preparations from seven dogs. Panel A illustrates the ability of different concentrations of BOL to decrease responses to 2 μg NE (o—o), but not to 10 mg KCl (x--x) (expressed as percent of control responses); B, dose–response curves to NE in the absence (o–o) and in the presence (x--x) of 1×10^{-6} M BOL; C, dose–response curves to KCl in the absence (o–o) or in the presence (x--x) of 1×10^{-6} M BOL. * Differs from control, $p<0.05$.

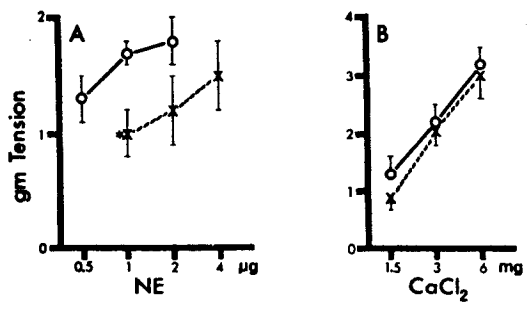

Fig. 2. Effects of NE and $CaCl_2$ on the contractile force of isolated hearts in the absence (o–o) or in the presence (x--x) of 1×10^{-6} M BOL. Each point represents the mean (± S.E.) of responses in six hearts. * Differs from control, $p<0.05$.

The effects of BOL on the vasoconstrictor responses to NE and to KCl are presented in fig. 1. Responses to 10 mg KCl (panel A) were not altered by BOL in concentrations up to 1×10^{-5} M; responses to 2 μg NE were reduced by concentrations of BOL from 1×10^{-7} M to 1×10^{-5} M. The dose–response curve to NE (panel B) was shifted to the right by BOL 1×10^{-6} M. BOL also significantly reduced the response to 25 mg but not lower doses of KCl (panel C). BOL (1×10^{-6} M) reduced the positive inotropic effects of NE in isolated hearts (fig. 2), but did not alter responses to $CaCl_2$ which does not activate cardiac beta adrenergic receptors. Because of limited supplies, LSD (1×10^{-6} M) was tested in only one isolated heart. It reduced responses to NE but not those to $CaCl_2$.

4. DISCUSSION

Once produced in the central nervous system by hormonal or electrical stimulation, cyclic AMP is capable of activating protein kinase, but its subsequent metabolic effects are unknown (Kakiuchi et al., 1969; Miyamoto et al., 1969; McAfee et al., 1971). The receptor properties of the adenyl cyclase system in the brain are rather diverse: in brain slices catecholamines, histamine, adenosine, and depolarizing agents such as batrachotoxin and veratridine are capable of stimulating cyclic AMP formation (Sattin and Rall, 1970; Shimizu et al., 1970; Palmer et al., 1971a). Several studies have demonstrated that β-adrenergic receptor blocking agents are capable of modifying the cyclic AMP response to certain adrenergic agents in the brain (Kakiuchi and Rall, 1968; Weiss and Costa, 1968) but some controversy exists about whether or not alpha receptor blocking agents can exert this effect. Recent data, however, suggest that the cyclic AMP response in the rat hypothalamus is effectively blocked by phentolamine and phenoxybenzamine, which are specific alpha receptor antagonists (Palmer et al., 1971a). The 5-hydroxytryptamine antagonists LSD and BOL have been shown not to be absolutely specific in their smooth muscle blocking actions (Gyermek, 1961). In the present study, LSD and BOL effectively antagonized the cyclic AMP elevation produced by NE in the rat hypothalamus and brain stem, and also antagonized adrenergic responses in

arteries and hearts mediated respectively by α- and β-adrenergic receptors. Thus, on the basis of present evidence, the receptor mechanism in the brain which mediates the adenyl cyclase response to the catecholamines cannot be rigorously described as either an α- or a β-adrenergic receptor. Cocaine, which has been shown to enhance peripheral alpha adrenergic responses to NE in concentrations of 10^{-5} M and below (Burks and Cooper, 1967), did not affect the cyclic AMP response to NE in the rat brain in vitro.

In conclusion the data suggest that some of the central and peripheral actions of LSD and BOL can include relatively nonspecific blockade of adrenergic receptors.

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