XYLAMINE ENHANCES PINEAL GLAND N-ACETYLTRANSFERASE ACTIVITY IN VITRO

KATHERINE A. HAAK, GERMÁN TORRES, PATRICIA L. GARVEY, DAVID M. BRONSTEIN, ARTHUR K. CHO and LOY D. LYTLLE

1Laboratory of Psychopharmacology, Department of Psychology, University of California, Santa Barbara, CA 93106,
2International Ophthalmic, Laguna Hills, CA 92651,
3Department of Psychiatry, Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109 and
4Department of Pharmacology, UCLA School of Medicine, Center for the Health Sciences, Los Angeles, CA 90024, U.S.A.

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Abstract—1. The action of N-2'-chloroethyl-N-ethyl-2-methyl benzylamine (xylamine) on rat pineal gland sympathetic innervation was examined.
2. This alkylating agent caused a concentration-dependent increase in pineal gland N-acetyltransferase (NAT) activity in neurologically intact pineal glands that was suppressed in glands previously subjected to bilateral superior cervical ganglionectomy.
3. Xylamine-induced elevations in NAT activity were attenuated by β-noradrenergic antagonist drugs but not by α-noradrenergic antagonist drugs.
4. Since pineal gland uptake of radiolabelled norepinephrine (NE) was impaired by xylamine, the drug may increase pineal gland NAT activity by inhibiting NE reuptake into the presynaptic nerve terminal, thereby increasing the amount of the neurotransmitter available to stimulate pinealocyte β-noradrenoceptors.

INTRODUCTION

Xylamine (N-2'-chloroethyl-N-ethyl-2-methyl benzylamine) appears to be an irreversible inhibitor of neuronal norepinephrine (NE) uptake in both central (Dudley et al., 1981) and peripheral nerves (Cho et al., 1980; Fischer and Cho, 1982; Ransom et al., 1984, 1985). The aziridinium ion, resulting from the intramolecular cyclization of xylamine, is believed to mediate this inhibitory effect on neuronal uptake by alkylating the NE uptake carrier molecule (Ransom et al., 1982, 1984). A great deal has been learned recently about the neurochemical bases of xylamine-induced alterations in noradrenergic neurotransmitter mechanisms, but relatively little is known about the possible functional consequences of these actions.

The present experiments were undertaken to study the possible functional effects of xylamine on a set of well-characterized noradrenergic synapses known to influence the synthesis of the pineal gland hormone melatonin. The pineal gland enzyme, serotonin N-acetyltransferase (NAT; EC 2.3.1.5) plays an important role in the formation of melatonin.

Norepinephrine molecules released from post-ganglionic, sympathetic neurons innervating the pineal gland enhance the activity of NAT via stimulation of a β-noradrenoceptor linked, cyclic AMP dependent mechanism (Axelrod, 1974). Pharmacological manipulations, including those involving such well known noradrenergic neurotransmitter uptake inhibitors as desmethylimipramine and amphetamine, that increase synaptic NE levels, elevate pineal gland NAT activity (Axelrod, 1974; Klein, 1979; Reiter, 1981). In the present experiments we determined whether xylamine might also increase pineal gland NAT activity via noradrenergic mechanisms similar to those known to be important for other indirectly acting noradrenergic neurotransmitter agonists.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley albino rats, weighing 250–350 g, were either purchased from Charles River Breeding Laboratories (Wilmington, MA) or bred and reared in our animal colony. Animals were housed in groups of 5–6 in hanging wire cages, given ad libitum access to tap water and laboratory chow (Ralston Purina, St Louis, MO), maintained under 22–23°C ambient temperature conditions at 55% relative humidity, and exposed to a 12:12 hr light:dark cycle (lights on at 0700 hr; incandescent illumination was approx. 32 lx during the light phase).

All experiments were conducted during the middle of the light phase of the cycle. Animals were killed by decapitation and pineal glands were excised within 1–2 min. Pineal glands were immediately placed in BGJb Fritton-Jackson culture medium (Grand Island Biological Co., Grand Island, NY) and blood vessels and connective tissue were removed with the aid of a dissection microscope prior to in vitro culture.

Chemicals

Xylamine HCl was synthesized as described by Kammerer et al. (1979). A 100 μM solution of xylamine HCl was

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prepared in 10 mM sodium phosphate buffer (pH 7.4) and
allowed to stand at least 30 min at room temperature prior to
use. Yohimbine HCl was purchased from Sigma Chemi-
cal Co. (St Louis, MO), and all other drugs used in these
experiments were generously provided by the following
companies: prazosin HCl (Pfizer Inc., Groton, CT); des-
methylimipramine HCl (DMI; Revlon Health Care Group,
Tuckahoe, NY), butoxamine HCl (Burroughs Wellcome
Co., Research Triangle Park, NC) and practolol HCl
(Imperial Chemical Industries Ltd, Alderley Park, Cheshire,
England).

Effect of xylamine on pineal gland and NAT activity

For in vitro experiments, pineal glands were placed in
organ culture using a modification (Altar et al., 1983) of the
method of Parfitt et al. (1976). In brief, pineal glands were
preincubated for 30 min in 247 µl of incubation medium at
37°C in a 95:5 O₂:CO₂ atmosphere. 13 µl of either vehicle
or one of several different concentrations of the drug were
added to the incubation medium and 4 hr later glands were
frozen and stored at -70°C.

Pineal gland NAT activity was measured using the proto-
col described by Altar et al. (1983) based on the method of
Deguchi and Axelrod (1972). Changes in pineal gland NAT
activity, calculated as the nmols of N-acetyltryptamine
formed per pineal per hr, were analyzed using analysis of
variance followed by post hoc Student's t-test.

Effect of xylamine on NAT activity in denervated pineal
glands

In order to determine whether the possible xylamine-
induced alterations in NAT activity were mediated by pre-
or post-synaptic mechanisms, pineal glands obtained from
denervated or neurologically intact animals were incubated
with vehicle or xylamine. Denervations were accomplished
by bilateral superior cervical ganglionectomies (SCGX)
performed under ether anesthesia 7 days prior to placing the
glands in organ culture. Successful removal of the ganglia
was assessed in vivo by the appearance of ptosis.

Xylamine inhibition of [³H]NE uptake

Previous studies indicated that xylamine effectively blocks
neural reuptake of NE (Cho et al., 1980; Ransom et al.,
1985). We determined whether xylamine might affect nor-
epinephrine uptake by using a modification of the method of
Bowers et al. (1984). Briefly, pineal glands were placed in
organ culture and coincubated with vehicle, 100 µM xylamine,
or the well-known NE reuptake inhibitor DMI (100 µM). After
a 1 hr preincubation period glands were subjected to two wells containing 1.2 × 10⁻³⁴ M [³H]-1-norepinephrine (specific activity = 19.7 Ci/mmol;
New England Nuclear Corp., Boston, MA) as well as the
appropriate drug or vehicle solution. At the end of the
15 min incubation period the glands were washed in Earle's
salt solution, and the amount of [³H]NE in the glands was
determined by liquid scintillation spectrometry.

Blockade of xylamine-induced increase in NAT activity

In addition to the importance of β-noradrenergic receptor
stimulation for pineal gland NAT activity, α-noradrener-
gic receptors may also play a permissive role in the noradrenergic
induction of the enzyme (Klein et al., 1983; Alphs and
Lovenberg, 1984). In order to more precisely characterize
the receptor subtype which might mediate xylamine-induced
changes in NAT activity, pineal glands were coincubated
with xylamine and 100 µM prazolol HCl or butoxamine
HCl (β₁- and β₂-noradrenocceptor antagonist drugs, re-
spectively) or prazosin HCl or yohimbine HCl (α₁- and
α₂-noradrenocceptor antagonist drugs, respectively).

RESULTS

Xylamine caused a concentration dependent in-
crease in pineal gland NAT activity (Fig. 1). At the
lowest concentration tested (5 µM) xylamine produced
a 2-fold increase in NAT activity whereas at its
highest concentration (100 µM) the activity of the
enzyme was elevated 77-fold. Although xylamine
increased NAT activity in neurologically intact pineal
glands, its in vitro action was substantially attenuated
in pineal glands obtained from animals previously
subjected to SCGX (Fig. 1).

Neurologically intact pineal glands took up
approximately 0.30 pmol of [³H]NE from the
incubation media during the 15 min test period.
Interestingly, both xylamine and DMI caused an
80-90% inhibition of [³H]NE uptake into these
glands (Fig. 2).

When administered alone none of the α- or
β-noradrenergic antagonist drugs produced any

Fig. 1. Effect of xylamine on in vitro NAT activity in intact
or SCGX denervated pineal glands placed in organ
culture. All values are means (±SEM) (N = 4–5
animals per group). The left panel represents the number
of pmol acetyltryptamine/pineal gland/hour in intact
rats (*P < 0.02; **P < 0.01; ***P < 0.001 compared to
intact vehicle treated control animals). The right panel
represents acetyltryptamine formation in pineal glands
from bilaterally superior cervical ganglionectomized (SCGX) rats
(***P < 0.001 as compared to intact xylamine treated
animals).

Fig. 2. Effects of xylamine or DMI on in vitro pineal gland
[³H]NE uptake. Pineal glands were incubated with [³H]NE
for 15 min in the presence of vehicle, xylamine or DMI. All
values are mean NE uptake (±SEM) (N = 5 animals per
animal).
Table 1. Effects of α- or β-noradrenoceptor antagonist drugs on xylamine induced increases in pineal gland NAT activity

<table>
<thead>
<tr>
<th>Drug condition</th>
<th>Vehicle</th>
<th>Xylamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAT activity (nmol/pineal/hr)</td>
<td>2.24 ± 0.05*</td>
<td>2.06 ± 0.03*</td>
</tr>
</tbody>
</table>

*P < 0.01 compared to vehicle control values

significant changes in pineal gland NAT activity compared to vehicle control values (Table 1). However, each of the β-noradrenoceptor but none of the α-noradrenoceptor antagonist drugs significantly attenuated the xylamine-induced elevations in pineal gland NAT activity.

DISCUSSION

Xylamine in vitro enhanced pineal gland NAT activity in a concentration dependent manner. This action of the drug is abolished in denervated glands and is attenuated by β- but not α-noradrenoceptor antagonist drugs. Since the abilities of neurologically intact pineal glands to accumulate [3H]NE are substantially reduced by xylamine, our combined data suggest that xylamine elevates pineal gland NAT activity by increasing synaptic NE concentrations at pinealocyte β-receptors via the inhibition of neurotransmitter uptake into postganglionic sympathetic nerve terminals. Previous studies suggest that rat vas deferens presynaptic noradrenergic nerve terminals accumulate xylamine (Ransom et al., 1985). Chemical denervation of the terminals by 6-hydroxydopamine depletes NE content by 85% and reduces radio labelled xylamine accumulation by 70%. Since the NAT response to xylamine is also abolished in denervated pineal glands, it appears that xylamine increases pineal gland NAT activity by acting presynaptically on one or more noradrenergic neurotransmitter mechanisms.

The NE uptake inhibiting properties of xylamine have been described previously in rabbit aortic rings (Cho et al., 1980) as well as in rat superior cervical ganglia (Fischer and Cho, 1982), vas deferens (Ransom et al., 1985) and cortical synaptosomes (Dudley et al., 1981). Our data indicate that xylamine also inhibits uptake of the neurotransmitter in postganglionic sympathetic terminals innervating the pineal gland. Nevertheless, these results do not rule out other possible mechanisms of action of xylamine. For example, xylamine and its chemical analogue N-[2-(chloroethyl)]-N-ethyl-2-bromobenzylamine (DSP4) appear to exert neurotoxic effects on peripheral and central noradrenergic nerve terminals (Dudley et al., 1981; Jaim-Etcheverry and Zieher, 1983). Xylamine-induced neurotoxic effects on peripheral catecholaminergic nerves still remain to be determined, however, since only marginal NE depletions in the rat vas deferens have been observed previously (Ransom et al., 1984, 1985). Nevertheless, xylamine might also increase synaptic NE by other nontoxic mechanisms, such as by increasing release of the neurotransmitter from sympathetic nerve terminals via an amphetamine-like action on the noradrenergic nerve terminals that adhere to the pineal gland when it is placed in organ culture.

Thus far xylamine has proven to be useful in examinations of the molecular mechanisms important for neuronal NE uptake. Although its neurochemical mechanisms of action appear to differ significantly from some of the other noradrenergic reuptake inhibiting drugs available in the experimental and clinical armamentarium, it remains to be determined whether these mechanisms will prove useful as a tool for gaining a better understanding of the functional importance of noradrenergic neurons in various in vivo or in vitro preparations.

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REFERENCES


