In summary, we have reported here that ABAL had a depressive effect on the motor activity of animals, and that the effect may be associated with the production of GABA from ABAL in brain. ABAL may serve as a therapeutic prodrug of GABA for some neurological disorders of the GABA system, though the problem of whether the physiological actions of GABA produced from ABAL are exactly equal to those of endogenous GABA has yet to be investigated thoroughly.

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Increase in serum corticosterone concentration and decrease in hypothalamic epinephrine concentration by *N*-propylnorapomorphine in rats

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N-n-Propylnorapomorphine (NPA) has been described to be a dopamine agonist with greater potency and slightly longer duration of action than apomorphine [1]. NPA has been shown to decrease dopamine turnover in brain and to reduce the firing rate of nigral dopamine neurons [2], to cause turning in rats with unilateral nigrostriatal lesions [3], to cause hypoactivity at low doses and hyperactivity at higher doses [2, 4], and to cause stereotypy in rats and mice [4, 5]. Recently we have described two additional effects of dopamine agonists in rats, namely elevation of serum corticosterone concentration [6] and lowering of hypothalamic epinephrine concentration [7]. Since neither of these effects has been reported for NPA, and since most of the dopamine agonists we studied previously were ergolines, we determined whether NPA had the ability to increase serum corticosterone concentration and decrease hypothalamic epinephrine concentration in rats.

Male Wistar rats weighing 150–200 g (from Harlan Industries, Cumberland, IN) were housed in groups of five in a 24° room with 12 hr light:dark cycles for at least 1 week prior to the experiment. All rats had free access to food and water during the experiments. Drugs were injected i.p. The injection volume was 1 ml/kg, control rats receiving vehicle at the same volume. (−)NPA hydrochloride (Research Biochemicals, Wayland, MA) was dissolved in distilled water, and spiperone (Janssen Pharmaceutica, Beerse, Belgium) was dissolved in 0.01 N HCl. Treated rats were decapitated, and blood collected from the trunk was allowed to clot. Serum obtained after centrifugation was stored at −15° prior to analysis. Whole brain or dissected brain regions were frozen on dry ice and stored at −15° prior to analysis. Corticosterone was measured spectrofluorometrically [8]. High performance liquid chromatography with electrochemical detection was used to measure epinephrine [9] and the dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) [10]. All results are shown as mean values ± standard errors for five rats per group. Comparisons between groups were made by Student's *t*-test.

NPA increased serum corticosterone concentration and decreased DOPAC and HVA concentrations in whole brain (Table 1). The 0.003 mg/kg dose had no significant effect, but 0.1 and 0.3 mg/kg doses increased corticosterone dose-dependently, the 1 mg/kg dose causing no further increase. DOPAC and HVA concentrations were decreased significantly at the 0.1 to 1 mg/kg doses of NPA.

Spiperone pretreatment increased whole brain concentrations of DOPAC and HVA but had no significant effect on serum corticosterone (Table 2). In spiperone-pretreated rats, the increase in serum corticosterone and the decrease in cerebral DOPAC and HVA caused by NPA in control rats were blocked completely.

Epinephrine concentration in hypothalamus was decreased significantly by NPA at a dose (1 mg/kg) that decreased striatal dopamine metabolites (Table 3). Spiperone pretreatment overrode the decrease in striatal DOPAC and HVA and prevented the decrease in hypothalamic epinephrine.

These findings reveal that NPA, like other dopamine agonists we have studied, decreased hypothalamic epinephrine [7] and increased serum corticosterone [6, 11]. That these actions of NPA were mediated by dopamine receptor activation is supported by the findings that these changes (1) occurred at NPA doses that decreased whole

Table 1. Dose-dependent effects of NPA on serum corticosterone concentration and on the concentration of dopamine metabolites in rat brain*

Dose of NPA (mg/kg, i.p.)	Serum corticosterone (μg/100 ml)	Dopamine metabolite concentration (nmoles/g whole brain) DOPAC	HVA
0	5.0 ± 0.4	0.42 ± 0.03	0.46 ± 0.02
0.03	12.2 ± 6.9	0.36 ± 0.01	0.39 ± 0.03
0.10	28.3 ± 4.1†	0.27 ± 0.02†	0.31 ± 0.02†
0.30	44.4 ± 3.2†	0.28 ± 0.01†	0.27 ± 0.01†
1.00	44.2 ± 5.1†	0.27 ± 0.01†	0.26 ± 0.01†

* NPA was injected 1 hr before rats were killed.
† Significant effect of NPA ($P < 0.01$).

Table 2. Antagonism by spiperone pretreatment of the effects of NPA on serum corticosterone and on dopamine metabolites in whole brain*

	Dose of NPA (mg/kg, i.p.)	Serum corticosterone (μg/100 ml)	Brain DOPAC (nmoles/g)	Brain HVA (nmoles/g)
Control	0	5.0 ± 0.5	0.44 ± 0.02	0.39 ± 0.03
	0.3	37.2 ± 5.8†	0.32 ± 0.01†	0.23 ± 0.02†
Spiperone-pretreated	0	7.4 ± 1.2	0.74 ± 0.13	0.63 ± 0.11
	0.3	8.2 ± 1.0	0.84 ± 0.15	0.67 ± 0.16

* NPA was injected 1 hr after spiperone (0.1 mg/kg, i.p.) and 1 hr before rats were killed.
† Significant effect of NPA ($P < 0.01$).

Table 3. Antagonism by spiperone of the lowering of hypothalamic epinephrine concentration and striatal dopamine metabolite concentrations by NPA*

Treatment group	Hypothalamic epinephrine (pmoles/g)	Striatal dopamine metabolites (nmoles/g) DOPAC	HVA
Control	140 ± 8	6.89 ± 0.58	4.30 ± 0.28
NPA	94 ± 7†	4.37 ± 0.33†	2.33 ± 0.11†
NPA + spiperone	128 ± 4†	15.27 ± 2.12†‡	10.32 ± 1.71†‡
Spiperone	127 ± 3	19.32 ± 1.58†	15.47 ± 1.49†

* NPA (1 mg/kg, i.p.) was injected 1 hr after spiperone (0.5 mg/kg, i.p.) and 2 hr before rats were killed.
† Significant difference from control group ($P < 0.05$).
‡ Significant difference from group with NPA alone ($P < 0.05$).

brain and striatal HVA and DOPAC, and (2) were prevented by pretreatment with spiperone, a dopamine antagonist. The decrease in hypothalamic epinephrine concentration by dopamine agonists has been shown not to be due to direct inhibition of norepinephrine *N*-methyltransferase ([7]; NPA at 10^{-6}–10^{3} M *in vitro* caused at most 20% inhibition of norepinephrine *N*-methyltransferase) and might instead be due to increased epinephrine release or to reduction in synthetic rate via some other mechanism. The increase in serum corticosterone concentration produced by dopamine agonists apparently results from a central action rather than any effect directly on the adrenal cortex, since the increase is blocked by centrally acting dopamine antagonists but not by domperidone, which blocks dopamine receptors in the periphery but not centrally [6, 11].

The relationship between the decrease in hypothalamic epinephrine concentration and the increase in serum corticosterone concentration produced by dopamine agonists, if any relationship exists, is not known. There are two general possibilities—the effects are separate, not interrelated, consequences of dopamine receptor activation, or one effect causes the other, that is, the effects on epinephrine cause the increase in corticosterone, or vice-versa. Our data do not permit a choice between those possibilities, but certain literature may be relevant. Roth *et al.* [12] have postulated that central epinephrine neurons are inhibitory to corticosterone release in the rat, based on the finding that two inhibitors of norepinephrine *N*-methyltransferase increased serum corticosterone concentration. Although they had no direct evidence that the corticosterone increases were caused by the inhibition of epinephrine synthesis in brain by these compounds, the possibility could be considered that NPA elevated corticosterone concentration in a related manner by reducing an inhibitory input from epinephrine neurons. Perhaps dopa-

mine neurons make synaptic contact with epinephrine neurons to modulate epinephrine synthesis and/or release, and NPA acts on the receptors at these synapses. This conclusion could not be made solely from the observations reported here, however. The effects of NPA and other dopamine agonists on hypothalamic epinephrine and corticosterone could be two separate consequences of a "stress" associated with activation of central dopamine receptors, since hypothalamic epinephrine can be depleted by stress [13] and, of course, corticosterone levels are increased by stress. Further studies will be needed to address the mechanism(s) by which dopamine agonists of diverse structural types decrease hypothalamic epinephrine and increase serum corticosterone concentration.

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Formycin B resistance in *Leishmania**

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There are a few curative agents for the diseases caused by pathogenic protozoa, and the emergence of drug-resistant organisms presents a serious problem. Further, since knowledge of how these organisms acquire drug-resistance is limited, there are no rational approaches to avoid or circumvent this problem. One objective of this laboratory has been to define molecular mechanisms of drug resistance in pathogenic protozoa. Our approach is to select strains of tissue culture forms of protozoa which are resistant to drugs having different modes of action and then to elucidate the biochemical basis of resistance.

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† Abbreviations: FoA, Formycin A; FoB, Formycin B; FoA-MP, FoA-DP, FoA-TP, Formycin A 5′-mono-, di- and triphosphates, respectively; FoB-MP, Formycin B 5′-monophosphate; DME, Dulbecco's modified Eagle's medium; TCA, Trichloroacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; and HPLC, high performance liquid chromatography.

Recently, we reported the selection of methotrexate-resistant *Leishmania tropica* and demonstrated that the mechanism of resistance involved overproduction of dihydrofolate reductase which was mediated by gene amplification [1]. In the present work, we describe the selection of *L. tropica* promastigotes which are highly resistant towards the nucleoside analog Formycin B (FoB)†. We show that the resistant organisms are impaired in their abilities to transport this drug and to accumulate the nucleotide metabolites believed to be responsible for cytotoxicity.

Materials and methods

Growth of organisms. *L. tropica* promastigotes (strain 252; Iran) obtained from S. Meshnick were grown at 26° in M199 medium (GIBCO, Grand Island, NY) supplemented with 20% fetal calf serum, 25 mM Hepes (pH 7.4), and gentamicin at 50 μg/ml or in Dulbecco's Modified Eagle's medium (GIBCO) supplemented with 10% fetal calf serum and 50 μg/ml gentamicin. For growth rate studies, cells were seeded at 1×10^6 cells/ml and counted daily using a Coulter Counter ZBI until cell growth entered stationary phase. The IC_{50} values refer to the concentration of drug that inhibited the growth rate by 50%.

Selection of FoB-resistant cells. FoB-resistant *L. tropica* promastigotes were obtained by using a stepwise selection