Table 2. Effect of adrenergic receptor blockade during incubation with norepinephrine on lipogenesis. Rat hepatocytes were incubated during 60 min with 3 μM NE without and with α- and β-receptor blockers, and with simultaneously added ^3H_2O (750 μmol) at 0 min.

	Lipogenesis (nmoles ^3H_2O incorporated into fatty acids per mg dry weight)	
	Control (n = 6)	Ne (3 μM) (n = 6)
NE		
without blockers	32.1 ± 7.7	21.7 ± 4.8*
with 10^{-7} M prazosin	29.9 ± 7.4	32.8 ± 5.9
with 10^{-8} M prazosin	33.8 ± 7.1	30.0 ± 6.6
with 10^{-9} M prazosin	32.8 ± 6.1	24.4 ± 3.9*
with $3 \cdot 10^{-5}$ M propanolol	34.7 ± 10.7	25.7 ± 6.7*
with 10^{-7} M yohimbine	34.8 ± 10.9	20.0 ± 3.2*

* $p < 0.05$ (Wilcoxon's test for matched pairs).

of lipogenesis and increase in ketogenesis and CO_2 production from long chain fatty acids.

Previous studies on the effect of norepinephrine on ketogenesis have yielded conflicting results. We observed recently a stimulatory effect of norepinephrine on 1-^{14}C palmitate conversion into ketone bodies and on net ketone body output by isolated hepatocytes, in the absence of changes in fatty acid uptake[1]. Ketogenic effects of norepinephrine were also observed by others[8], but not during different experimental conditions[9,10]. Regarding lipogenesis, Ly et al.[4] reported previously inhibition of fatty acid synthesis by norepinephrine in hepatocytes. In view of these existing data, it appeared of interest to examine the question whether stimulatory effects of norepinephrine, and inhibition of lipogenesis, can be detected simultaneously during the same incubation. The antilipogenic effect of norepinephrine has been demonstrated to be associated with decreased activity of acetyl-CoA carboxylase[4]. This enzyme enhances the conversion of acetyl-CoA into malonyl-CoA, suggesting that malonyl-CoA concentrations decreased during the present incubations with norepinephrine. Since malonyl-CoA is an important inhibitor of carnitine palmitoyl transferase I[5,11], it is likely that a decrease in the malonyl-CoA content was the reason for the observed stimulation of ketogenesis. The assumption of a common underlying mechanism is further supported by the fact that the effects of norepinephrine on ketogenesis and lipogenesis were both α_1-adrenergic[1].

Similar[1] to α_1-receptor activation, glucagon has been reported to exert antagonistic effects on lipogenesis and ketogenesis[12,13]. However, glucagon and α-adrenergic agonists have different modes of action. Glucagon exerted a stimulatory effect on gluconeogenesis[13,14] and inhibited acetyl-CoA carboxylase and lipogenesis[15] via cAMP-dependent pathways. In contrast, α-adrenergic activation of gluconeogenesis and of glycogenolysis[14] was cAMP-independent. α_1-Receptor activation has been reported even to decrease intracellular cAMP levels while increasing gluconeogenesis[16]. α-Adrenergic activation of hepatic fatty acid oxidation[9] and lipogenesis[4] were Ca^{++}-dependent.

Thus, the present studies demonstrate that simulation of ketogenesis by norepinephrine is associated with inhibition of lipogenesis. The data support the concept that α-adrenergic activation exerts a coordinated effect on the metabolism of hepatic fatty acids; enhancing their catabolism and decreasing their synthesis.

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0014-4754/87/101104-03$1.50 + 0.20/0

Sympatho-inhibitory mechanisms acting at sympathetic ganglia to attenuate hypothalamic-induced pressor effect in the cat

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Summary. Pressor and tachycardic effects induced in the cat by stimulation of a lateral hypothalamic (LH) site, are shown to be mediated by sympathetic ganglia nicotinic receptor, and potentiated under atropine methyl nitrate sympathetic ganglia blockage. It is postulated that a sympatho-inhibitory pathway muscarinic ganglionic mechanism, co-activated by the LH stimulation, attenuates the pressor and tachycardic effects, the potentiation presumably being a manifestation of blockage of that mechanism.

Key words. Lateral hypothalamus; descending sympatho-excitatory and sympatho-inhibitory pathways; muscarinic ganglionic receptor; attenuation of pressor effect; ganglionic inhibition in autonomic function.

Electrical stimulation of the cat lateral hypothalamus (LH) perifornical region, medially to the nucleus of the Fields of Forel induces moderate pressor effects, uniquely associated with an increase in or no change in heart rate (HR)[1,6]. Such a coincidence suggests suppression of the baroreceptor mechanisms[7]. An in depth study of this phenomenon provided significant clues on relationship of these changes to dysautonomic disturbances[3]. A potentiation of the pressor and

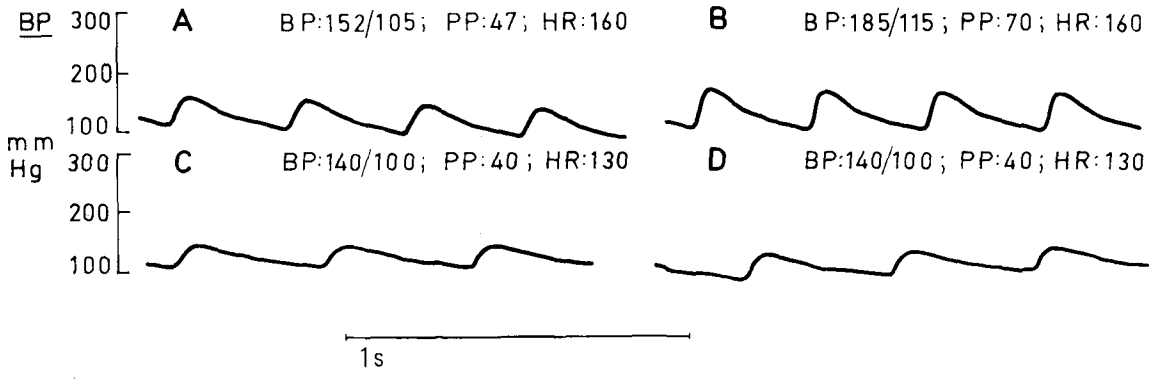

Figure 1. Blockage of LH induced pressor effect by systemic administration of 5 mg/kg hexamethonium. *A* Control; *B* effects of LH stimulation. Note the small rise in BP and pulse pressure (PP), HR remains unchanged; *C* A control record 10 min after 5 mg/kg HM. Note small drop in BP, PP and HR; *D* Blockage by HM of the effects of LH stimulation on BP, HR, and PP.

tachycardic effects that was observed under blockage of sympathetic ganglia by atropine methyl nitrate (ATMN) suggested that interactions take place at sympathetic ganglia between sympatho-inhibitory[2, 5] and sympatho-excitatory pathways, the latter, mediators of the pressor and tachycardic effects. These interactions assumed to be involved in the phenomenon under consideration are proposed to be normally active in attenuation of blood pressure (BP) and HR changes the blockage of which is assumed to be responsible for the potentiation observed.

The study was carried out on 28 locally grown Swiss breed cats, using ganglionic blocking agents. Each experiment was carried out in two stages a week apart, under α-chloralose anesthesia (55–65 mg/kg). Asepsis was maintained by a 3-day daily treatment of the animals with antiseptics and antibiotics. Arterial BP and ECG in lead II were recorded as reported previously[1]. Initially, baseline BP and HR were recorded as controls for the stability of the recordings.

The type of stimulation electrodes, the technique of implantation and the stimulation parameters used, were as previously reported[1]. Actual electrode placements were verified post mortem by histological techniques, including the use of the well-known Hess method.

Neurotransmission was investigated at the stellate ganglia with the use of peripherally acting ganglionic blocking agents. In the first stage ATMN (0.2 mg/kg) or the nicotinic blocking agent hexamethonium (HM; 1 mg/kg) were administered systemically 15 min prior to the LH stimulation. Stimulation electrodes were then installed chronically at the site inducing the desired effects. In the second stage the animals were artificially ventilated and the blocking agent ATMN (50 μg) or HM (1 mg) were applied directly to the in situ, microsurgically exposed and desheathed stellate ganglion of the ipsilateral or of both sides, about 4 min prior to LH stimulation. The direct drug application was carried out by placement of the agent into a paraffin bath in which the ganglion was submerged. Because unilateral stimulation induced ipsi – as well as bilateral effects, the agents were applied to the ipsilateral left stellate ganglion in eight cats and bilaterally in 10 cats. In 4 cats ATMN was reapplied after recovery from potentiation to the completely disconnected ganglion in situ. This, to control the possibility that the drug leaked out to produce the potentiation effect at a site away from the point of direct application. Four cats were bilaterally vagotomized to test the relationship of the vagus to the LH-induced effects.

To determine whether the mediation of the hypothalamic induced pressor and tachycardic effects is through the adrenals or the sympathetic ganglia, the blocking agent HM (5 mg/kg) was administered systemically to 6 cats, or 1 mg/kg

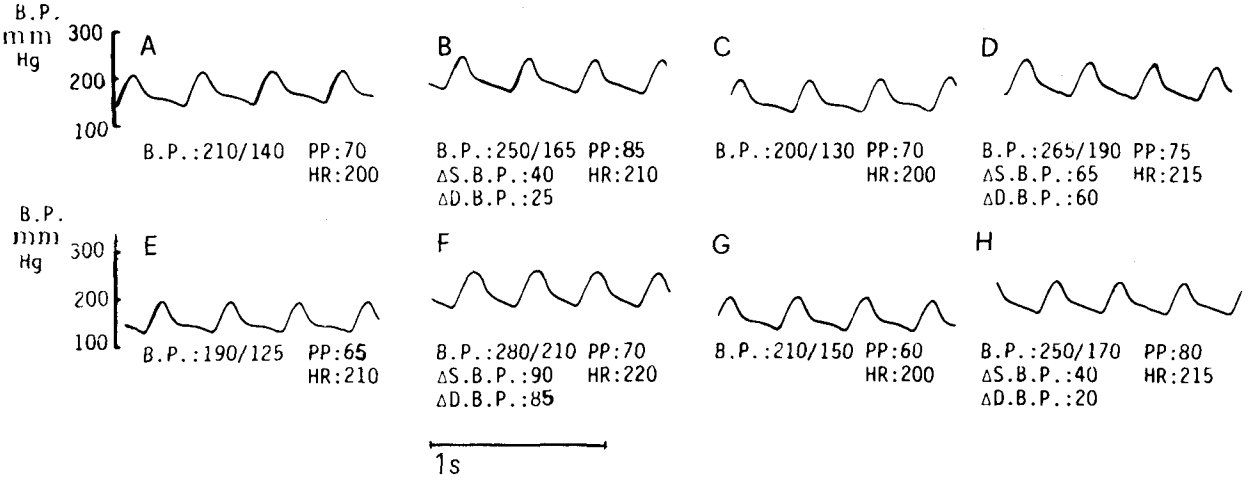

Figure 2. Illustrating the potentiation of the LH-induced pressor and tachycardic effects by systemic administration of 0.2 mg/kg ATMN. Corresponding values of BP, PP and HR are shown. *A* Control record; *B* LH stimulation: BP = 40/25 mmHg; PP = 15; HR = 10; *C* Control record 10 min after i.v. ATMN; *D* Effects of LH stimulation 10 min after ATMN. Potentiation: BP = 65/60 mmHg. No change in PP but HR = 15; *E* Control record 15 min after i.v. ATMN; *F* LH stimulation – further potentiation: BP = 90/85, PP unchanged, HR shows no potentiation; *G* Control at 1.5 h after ATMN. *H* LH stimulation – note that the BP potentiation is fading off.

Potentiation of LH-induced pressor response (1) by systemic 0.2 mg/kg ATMN and (2) by direct application of 50 µg ATMN to the ganglion at maximal drug effect

	Control mean BP (mm Hg)	LH stim. mean BP (mm Hg)	Mean of the differences between BP before and after LH stimulation	Control mean BP after ATMN (mm Hg)	LH stim. after ATMN, mean BP (mm Hg)	Mean of the differences between Δ BP before and after ATMN administration	p Values for the difference of means of BP
SBP	153 ± 8	192 ± 8	39 ± 5	145 ± 7	210 ± 9	20 ± 6	0.0009
(1) DBP	106 ± 7	132 ± 7	26 ± 4	102 ± 6	150 ± 7	25 ± 5	0.0003
							18 animals
SBP	152 ± 79.5	173 ± 31*	35 ± 19	143 ± 63	197 ± 46	40 ± 17	$p < 0.0001$
(2) DBP	103.6 ± 36	127 ± 26	37 ± 25	101 ± 62	144 ± 42	25 ± 10	$p < 0.0001$
							8 animals

* A smaller Δ BP induced by LH stimulation due to microsurgical manipulation of the ganglia.

was applied directly to the stellate ganglion, in 2 cats ipsilaterally, and in 2 cats bilaterally. As this totally blocked the pressor and tachycardic effects for over 4 h, as illustrated in figure 1 for the systemic HM administration, it is concluded that these pressor and tachycardic effects are mediated at the stellate ganglia by a nicotinic receptor[4].

Figure 2 illustrates from a sample cat the potentiation of LH-induced BP and HR produced by systemic pre-treatment with ATMN (0.2 mg/kg). In the table, a similar effect is shown by 18 cats administered ATMN systemically and by 7 out of 8 cats which received direct application of 50 µg ATMN to the stellate ganglion. It is noteworthy that the latter potentiation was obtained upon unilateral application of ATMN to a single ipsilateral stellate ganglion though other data obtained (Blum et al., unpublished) show that ATMN bilateral ganglionic application is more effective. In either systemic or direct mode of ATMN administration, the potentiation lasted for 3–4 h, recovery beginning in about 1½ h. We observed a mean difference of $29 \mp 6/25 \mp 5$ mmHg between LH stimulation induced control pressor effect and the pressor effect obtained by the same type of stimulation 15 min after systemic ATMN with $p = 0.0009$ for the potentiation of the Δ SBP and $p = 0.0003$ for the potentiation of Δ DBP at their maxima. We also observed a mean difference of $40 \mp 17/25 \mp 10$ mmHg between the LH-induced pressor effects after and before the direct local application of ATMN to the ganglia with $p = 0.0001$ for the potentiation of either Δ SBP or Δ DBP.

Four control experiments were performed to rule out the possibility of the blocking agents applied to the ganglia leaking out to produce the effects via the target organs. ATMN was re-applied to the ganglion in situ after recovery from potentiation and complete ganglionic disconnection. In two such cats there was no effect neither following the application nor the stimulation, indicating that this ganglion was the site of the drug action and also crucially involved in the generation of the pressor and tachycardic effects. In the other two cats there were weaker pressor and tachycardic effects induced by the stimulation which proved partial involvement of this ganglion in these effects. However, the application of the blocking agent after the disconnection produced no changes. We interpreted this to mean that the drug action was at this ganglion and not at a site peripherally to it.

Figure 3 demarcates LH sites where stimulation induced typical pressor and the tachycardic effects, with the respective potentiation magnitudes also shown. These data show a gradient for the potentiation with highest values around Fr.9, L. 2 and D.-2.5 and tapering off towards Fr.8.5 and 9.5. In four control vagotomized cats LH stimulation induced smaller pressor and tachycardic effects. This suggests that sympathetic cardiovascular function is best with the vagus intact.

While rises in BP and HR following LH stimulation prior to ATMN administration are small and possibly within physio-

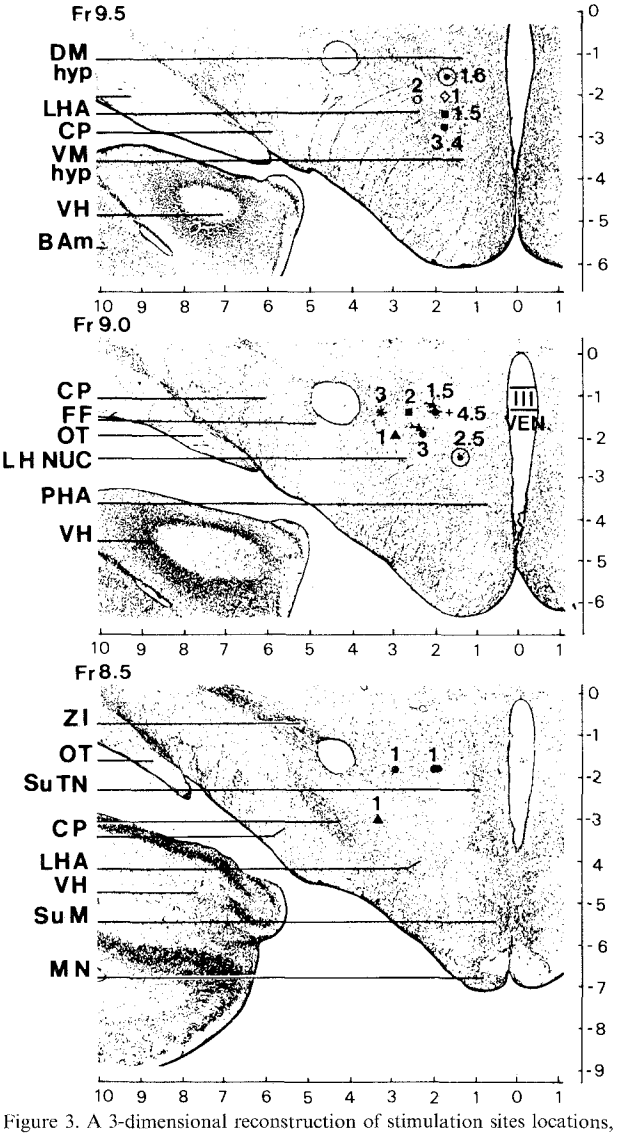

Figure 3. A 3-dimensional reconstruction of stimulation sites locations, based on histological sections at Frontal (Fr) 9.5, 9.0 and 8.5 of Horsely – Clarke (H–C) coordinates. Note that the H–C horizontal zero marked in the figure is 10 mm above the intra-aural line. The potentiation values obtained at each site are indicated. Stereotaxic scales are in mm. Abbreviations: DM hyp, dorsomedial hypothalamus; LHA lateral hypothalamic area; CP, cerebral peduncle; VM hyp, ventromedial hypothalamus; VH, ventral hippocampus; III VEN, third ventricle; B Am, Basal Amygdaloid; FF, nucleus of the fields of Forel; OT, optic tract; LH Nuc, lateral hypothalamic nucleus; PHA, posterior hypothalamic area; ZI, zona incerta; Su TN, Subthalamic nucleus; Su M, supra mammillary nucleus; MN, mammillary nucleus.

logical limits[8] when potentiated these may reach dysautonomic magnitudes. An attenuation mechanism is assumed to protect against such changes. We assume that a sympatho-inhibitory mechanism[2,5], co-activated by the LH stimulation together with sympatho-excitatory mechanism is responsible for attenuating the pressor and tachycardic effects, whereas blockage of this mechanism by ATMN results in the potentiation observed. If the sympatho-excitatory mechanism alone is activated, no potentiation is observable.

Descending in parallel from the lateral hypothalamus the excitatory and inhibitory pathways may interact at many levels, the potentiation phenomenon reported here being a sympathetic ganglia manifestation of such interactions. Furthermore, this sympatho-inhibitory mechanism may be identical with the slow muscarinic IPSP described by Libet et al.[9] at the sympathetic ganglia.

Acknowledgement. We are indebted to the Roboz Surgical Instrument Co, Wash. D.C. for material support and to Ilana Gelernter of the Applied Math. Dept for statistical aid.

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0014-4754/87/101106-04$1.50 + 0.20/0

Dopamine synthesis in rat striatum: Mobilization of tyrosine from non-dopaminergic cells

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24 February 1987

Summary. Unilateral nigrostriatal lesions in rats that almost totally depleted striatal dopamine had no effect on striatal levels of dopamine's precursor, tyrosine, nor on those of leucine. Since prolonged electrical stimulation of the slices markedly depletes them of tyrosine (1, 2) we conclude that tyrosine can be mobilized from non-dopaminergic striatal cells to augment dopamine release.
Key words. Tyrosine; dopamine, striatum; 6-hydroxydopamine lesions.

The relationship between tyrosine availability and catecholamine synthesis and release has been the subject of recent review[1]. Dopaminergic nerve terminals in electrically-stimulated rat striatal slices rapidly decrease their evoked output of transmitter when the exogenous supply of tyrosine is limiting; concomitantly, tissue tyrosine levels decline markedly whereas levels of other large, neutral amino acids of the same class as tyrosine remain unchanged, and those of dopamine itself fall only slightly[2].

As dopamine neurons comprise only a small proportion of the total cellular mass of the striatum, it therefore seemed unlikely that the tyrosine loss was restricted to these neurons unless their concentrations of the free amino acid were far in excess of those contained in the non-dopamine cells. To explore this question, dopamine nerve terminals were unilaterally destroyed in striata of rats by administration of the neurotoxin 6-hydroxydopamine, and residual tyrosine levels, measured in the lesioned tissue, were compared with levels in the intact contralateral striatum.

Methods. Male Sprague rats (150–200 g) were housed in pairs for at least one week, with food and water supplied ad libitum. Unilateral nigrostriatal lesions were produced by local injection of 6-hydroxydopamine (8 µg/2 µl saline containing 1 mg/ml ascorbic acid) into the anterior substantia nigra of animals maintained under light ether anesthesia. The stereotaxic coordinates used were A-2.4, V-1.6 and L-2.6, according to the atlas of Konig and Klippel[3]. Parglyine (75 mg/kg) was administered i.p. 60 min prior to the injection of 6-hydroxydopamine, in order to prolong the action of the neurotoxin. Animals were decapitated 2 weeks after placement of the lesions, and their striata assayed for dopamine, tyrosine and leucine by methods previously described[2]. Briefly, each demi-striatum was weighed, sonicated in 1 ml of perchloric acid (0.4 M) and centrifuged at 12,000 × g for 20 min. Dopamine was extracted from the supernatant fraction by adsorption to activated alumina, and eluted with acetic acid (0.75 M) using dihydroxybenzylamine (DHBA) as internal standard. Quantitation was by HPLC with electrochemical detection. Dopamine values were corrected for incomplete recovery (80–85%) by comparing the relative peak heights of dopamine and DHBA standards, passed over alumina, with those of the samples. Amino acids were analyzed by the HPLC method of Fernstrom and Fernstrom[4].

Results and discussion. Dopamine, tyrosine and leucine were measured in the left and right striata of rats with right substantia nigra lesions. Data are presented only from animals in which 90% or more of the nigrostriatal fibers were destroyed, as estimated from residual dopamine levels. In lesioned striata, dopamine levels decreased by more than 95% after administration of 6-hydroxydopamine (table). When tyrosine and leucine levels in lesioned and control tissues were compared, no significant differences were observed (table). Levels of leucine were monitored as an additional control because although this amino acid shares certain bio-

Tyrosine and leucine levels in striata of rats with unilateral 6-hydroxydopamine lesions

Amino acid	Lesioned striata (nmol/g)	Contralateral striata (nmol/g)	p
Tyrosine	106.5 ± 19.1	83.9 ± 16.2	NS
Leucine	36.7 ± 16.5	37.4 ± 6.2	NS
Dopamine	1.8 ± 0.5	78.5 ± 4.7	< 0.001

Values expressed are mean ± SEM for 5 animals.