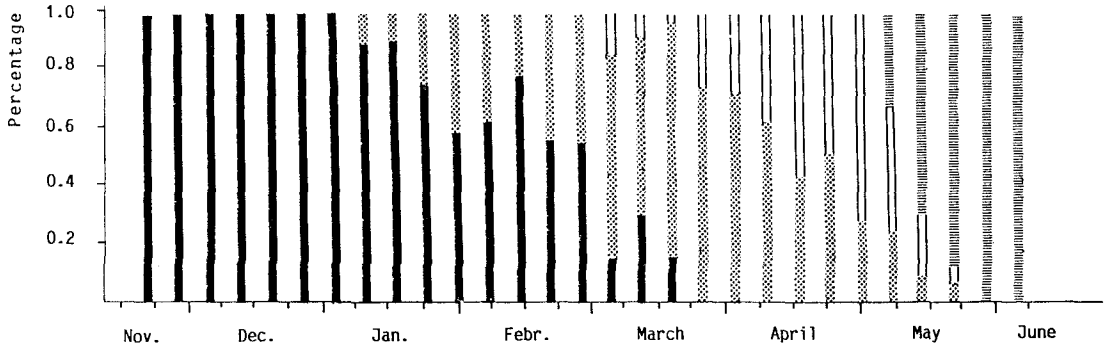

Figure 2. Development of imaginal wing discs in a field-grown population of European corn borer in Versailles (1984–1985). Each data point represents the dissection of 10–15 borers (■: I, II, III1 stages; ▨: III2 stage; □: III3 stage; ▤: III4 and more differentiated stages).

Application. By applying our results, homogeneous batches of larvae that are able to resume development simultaneously with a minimum of 15 days until pupation can be obtained throughout the year. This procedure leads to the completion of diapause development (100% of the wing discs are in the III3 stage). After 3 weeks at 25 °C and 12L:12D, diapausing larvae are kept on moistened paper strips, at 4 °C, under the same photoperiod, for 6 weeks. Then they are transferred again to 25 °C and 12L:12D conditions and deprived of water, for a period of 2 weeks. This procedure can be performed at any time of the year.

Conclusion. In the European corn borer, we show that morphogenesis of the imaginal wing discs occurs during diapause. The progress of wing disc diapause development is an irreversible and cumulative phenomenon; features corresponding to some of the characteristics pointed out by Beck and Alexander[9] in their definition of diapause development. Diapause termination occurs in favorable conditions when the wing discs of the whole population have terminated their own diapause development. In the wild strains of European corn borer, the examination of wing discs in winter gives a good idea of the potential ability for resumption of development of the larvae. Such indications are used to improve the modelling of population dynamics[12].

In our experimental conditions, the proposed 3 physiological criteria failed to reveal the 2 successive periods of diapause, maintenance period and transitional period, as proposed in Tauber and al.[13]. Additional studies thus need to be carried out to define, generalize or limit the physiological interest of our proposed criteria and particularly the wing disc development during diapause.

1 Beck, S. D., and Hanec, S. W., J. Insect Physiol. *4* (1960) 304.
2 Guennelon, G., and Audemard, H., Ann. Epiphyties *11* (1960) 337.
3 Gadenne, C., Lavenseau, L., and Peypelut, L., Acta oecol. appl. *7* (1986) 215.
4 Lavenseau, L., and Trabelsi, M., Agronomie *3* (1983) 523.
5 Andrewartha, H. G., Biol. Rev. *27* (1952) 50.
6 Tauber, M. J., and Tauber, C. A., Search (Agric.) Cornell Univ. Agr. Exp. Sta., Ithaca *3* (1973) 1.
7 Tauber, M. J., Tauber, C. A., and Masaki, S., Seasonal Adaptations of Insects, p. 411. Oxford Univ. Press, Oxford 1986.
8 Chambon, J. P., Bull. Soc. ent. Fr. *79* (1974) 105.
9 Beck, S. D., and Alexander, N., Biol. Bull. *126* (1964) 175.
10 McLeod, D. G. R., and Beck, S. D., Biol. Bull. *124* (1963) 84.
11 Beck, S. D., J. Insect Physiol. *13* (1967) 739.
12 Gadenne, C., Got, B., Peypelut, L., and Lavenseau, L., Agronomie (1987) in press.
13 Tauber, M. J., Tauber, C. A., and Masaki, S., in: Ecological Entomology, p. 658. Eds C. B. Huffaker and R. L. Rabb. John Wiley and Sons, New York 1984.

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α_1-Adrenergic stimulation of ketogenesis and fatty acid oxidation is associated with inhibition of lipogenesis in rat hepatocytes

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Summary. The effect of norepinephrine on fatty acid synthesis (^3H_2O incorporation into fatty acids), on fatty acid oxidation to CO_2 and on ketogenesis was studied in isolated hepatocytes of fed rats. After incubation with norepinephrine (50 μM), lipogenesis was lower (5.7 ± 1.1 nmoles ^3H_2O incorporated into fatty acids/mg dry weight/30 min) than in controls (7.5 ± 1.7; $n = 6$, $p < 0.02$). In contrast, (1-^{14}C) palmitate conversion into total ketone bodies was increased to 10.9 ± 1.8 nmoles/mg/30 min with norepinephrine, vs 8.5 ± 1.6 in controls ($p < 0.05$), and more (1-^{14}C) palmitate was converted to $^{14}CO_2$ with norepinephrine than in controls (1.48 ± 0.10 nmoles/mg/30 min vs 1.06 ± 0.11, $p < 0.05$). The inhibitory effect of norepinephrine on lipogenesis was abolished by addition of the α_1-receptor blocker prazosin, but not by α_2 or β-blockers. The results demonstrate that the ketogenic effect of norepinephrine is coupled with an inhibitory effect on lipogenesis which may be explained by diminished activity of acetyl-CoA carboxylase, diminished formation of malonyl-CoA and decreased activity of carnitine palmitoyl transferase I.

Key words. Catecholamines; norepinephrine; ketogenesis; fatty acid oxidation; fatty acid synthesis; prazosin; propranolol; yohimbine.

Recent studies demonstrated that norepinephrine increased oxidation of (1-^{14}C)-palmitate[1] or (1-^{14}C)-oleate[2] to $^{14}CO_2$ in isolated rat hepatocytes. This effect was inhibited by α-receptor blockers, and was Ca^{2+}-dependent[2]. The norepinephrine effect on fatty acid oxidation was associated with increased ketogenesis and diminished fatty acid esterification[1]. Since ketogenesis form 1-^{14}C-octanoate was not affected this suggested that norepinephrine influenced transport of long chain fatty acids into mitochondria, presumably by increasing the activity of carnitine palmitoyl transferase I[3]. A recent study demonstrated that norepinephrine decreased lipogenesis and the activity of acetyl-CoA carboxylase in isolated rat hepatocytes[4]. This effect may in turn explain norepinephrine's ketogenic effect since decreased lipogenesis results in diminished malonyl-CoA concentration, an important inhibitor of ketogenesis[5]. Therefore, the present experiments were performed to assess whether ketogenic effects of norepinephrine can be demonstrated simultaneously with inhibitory influences on lipogenesis using the same experimental conditions. In addition, the receptor involved in mediating norepinephrine's effect on lipogenesis was investigated.

Methods. Isolation and incubation of rat hepatocytes. Experiments were performed in male Wistar rats weighing 200–250 g and having free access to chow (Nafag pellets, Switzerland). Liver cells were prepared between 10.00 and 11.00 h by collagenase disgestion of the perfused liver as described[6]. All buffers for cell preparation contained 20 mM glucose to prevent glycogenolysis. After isolation the hepatocytes were incubated for 20 min in Krebs-Ringer bicarbonate buffer, containing 10 mM glucose 2.5 mM Ca^{++}, and 1 g/dl defatted bovine serum albumin. Viability of the hepatocytes was determined by trypan blue exclusion which indicated that more than 85% of the cells were viable. Approximately 20 aliquots of 2.25 ml of the liver cell suspension (8 mg/ml dry) were added to Erlenmeyer flasks (25 ml), and incubated at 37 °C in a shaking waterbath under an atmosphere of 95% O_2 and 5% CO_2. Norepinephrine was prepared for incubation[1] at concentrations of 50 and 5 μM. In the incubations with 50 μM norepinephrine (1-^{14}C)-palmitate at a final concentration of 0.5 mM, or 750 μM ^3H_2O, was added during the last 30 min of incubation. In the studies using 5 μM norepinephrine the tracers were added simultaneously with the catecholamine. (1-^{14}C)-palmitate-albumin complexes were prepared according to Spector and Hoak[7] using defatted serum albumin. Paired incubations without and with norepinephrine were performed using cell suspensions from the same livers.

Analyses. $^{14}CO_2$ was liberated in aliquots of liver cells by addition of 0.3 ml perchloric acid (30%) injected through the rubber stoppers of the flasks. This was repeated every 30 min on different aliquots of the liver cell suspension. After gassing for 30 min with CO_2-free air, $^{14}CO_2$ was trapped in a scintillation vial containing a CO_2-absorbing counting cocktail (CO_2-Oxyfluor®). Perchloric acid extracts were gassed with N_2 to remove $^{14}CO_2$. The remaining acid-soluble products represented labeled ketone bodies[1] and were counted in a β-counter. ^{3}H-labeled fatty acids were extracted according to McGarry et al.[5]. In brief, 0.75 ml 20% KOH and 7 ml ethanol were added to the incubation media, and fatty acids were saponified during 2 h at 70 °C in a waterbath. The unsaponified fatty acids were extracted with 5 ml petrol ether and discarded. Thereafter, the ^{3}H-labeled fatty acids were acidified with 1 ml 10 N HCl and extracted 3 times with 4 ml hexane. The hexane extracts containing fatty acids were washed 3 times with 2 ml H_2O; after addition of 3 ml benzole, they were dried overnight with N_2, and counted after adding 10 ml Biofluor®.

Materials. Collagenase, yohimbine HCl and DL-propranolol were from Sigma, St. Louis, USA; prazosin was from Pfizer, Switzerland; defatted serum albumin and norepinephrine-HCl from Fluka, Switzerland. Palmitic acid was from Merck, FRG. Tracers and scintillation cocktails were from New England Nuclear, Boston, Mass.

Calculations. Conversion of (1-^{14}C) -palmitate into $^{14}CO_2$ and ^{14}C total ketone bodies, and incorporation of ^3H_2O into fatty acids was calculated by dividing the radioactivity content of these products by the specific activity of the ^{14}C-labeled ^{3}H-labeled fatty acids. Statistical analysis was performed using the Wilcoxon test for matched pairs. Data are means ± SEM.

Results. Effect of norepinephrine on ketogenesis, fatty acid oxidation to CO_2 and lipogenesis. Incubations were performed using norepinephrine at 50 μM, in analogy to our previous study[1]. Table 1 shows that norepinephrine increased the conversion of (1-^{14}C)-palmitate into CO_2 and total ketone bodies significantly ($p < 0.05$) after 90 min of incubation. Comparing for 30 with incubation for 90 min, (^{14}C) palmitate conversion into CO_2 was found to be already maximal after 30 min, whereas ketogenesis increased further between 30 and 90 min. Ketone body production increased, and CO_2 production decreased in control studies as incubation was prolonged from 30 to 90 min. The reason for this time effect was probably aging of the cells. Using similar incubation conditions, norepinephrine (50 μM) diminished lipogenesis after 60 min from 7.5 ± 1.7 to 5.7 ± 1.1 nmoles ^3H_2O incorporated into fatty acids/mg dry/30 min in controls, ($n = 6$, $p < 0.02$). After 90 min, the effect of norepinephrine on lipogenesis was of similar magnitude (21% decrease, $p < 0.05$). The relatively large variability of the data was due to differences between individual livers, but the effects were statistically significant on paired analysis.

Influence of adrenergic blockers on the effect of norepinephrine on lipogenesis (Table 2). These experiments were performed with a lower dose of norepinephrine (3 μM) in order to examine the question whether lower concentrations of norepinephrine also inhibited lipogenesis. Incubation of rat hepatocytes during 60 min with norepinephrine resulted again in significantly diminished lipogenesis (33% decrease). Control incubations demonstrated higher rates of lipogenesis after 60 min than in the studies with 50μM norepinephrine, probably because incubation with the tracer was shorter (see methods) compared to 30 min incubation (table 1). Addition of the α_1-receptor-blocker prazosin diminished dose-dependently the inhibitory effect of norepinephrine on lipogenesis in such a way that blockade of the norepinephrine effect at 10^{-8} M prazosin was partial, and at 10^{-7} M complete. In comparison, the β-blocker propranolol and the α_2-blocker yohimbine were ineffective.

Discussion. The present studies demonstrate that incubation of rat hepatocytes with norepinephrine resulted in a decrease

Table 1. Effect of norepinephrine on hepatic fatty acid oxidation and ketogenesis. Hepatocytes were incubated for 30 and 90 min with and without norepinephrine (50 μM); 1-^{14}C palmitate (0.5 mM) was added during the last 30 min of incubation.

	Incubation 30 min		Incubation 90 min	
	Control ($n = 6$)	NE ($n = 6$)	Control ($n = 6$)	NE ($n = 6$)
^{14}C-Palmitate conversion into CO_2[+]	1.51 ± 0.17	1.79 ± 0.8*	1.06 ± 0.11	1.48 ± 0.10*
^{14}C-Palmitat conversion into ketone bodies[+]	6.64 ± 1.15	7.10 ± 0.99	8.51 ± 1.64	10.92 ± 1.84*

[+] Expressed as nmoles/mg dry weight/30 min (means ± SEM); * $p < 0.05$ vs controls.

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.

1 Oberhänsli, R.D., and Keller, U., Diabetes *34* (1985) 774.
2 Sugden, M.C., Tordoff, A.F.C., Ilic, V., and Williamson, D.H., FEBS Lett. *120* (1980) 80.
3 McGarry, J.D., and Foster, D.W., Rev. Biochem. *49* (1980) 395.
4 Ly, S., und Kim, K., J. biol. Chem. *256* (1981) 11585.
5 McGarry, J.D., Takabayashe, Y., and Foster, D.W., J. biol. Chem. *253* (1978) 8294.
6 Seglen, P.O., in: Methods in Cell Biology, pp. 29–83. Ed. D.M. Presott. Academic Press, New York 1976.
7 Spector, A.A., and Hoak, J.C., Analyt. Biochem. *32* (1969) 297.
8 Kosugi, K., Harano, Y., Nakano, T., Suzuki, M., Kashiwagi, A., and Shigeta, Y., Metabolism *32* (1983) 1081.
9 Sugden, M.C., and Watts, D.I., Biochem. J. *212* (1983) 85.
10 Bahnsen, M., Burrin, J.M., Johnston, D.G., Pernet, A., Walker, M., and Alberti, K.G., Am. J. Physiol. *247* (1984) E173.
11 Cook, G.A., King, M.T., and Veech, R.L., J. biol. Chem. *253* (1978) 2529.
12 Witters, L.A., Moriarity, D., and Martin D.B., J. biol. Chem. 254 (1979) 6644.
13 Heimberg, M., Weinstein, I., and Kohout, M.J., J. biol. Chem. *244* (1969) 5131.
14 Cherrington, A.D., Assimacopoulos, F.D., Haper, S.C., Corbin, J.D., Park, C.R., and Exton, J.H, J. biol. Chem. *251* (1976) 5209.
15 Witters, L.A., Kowaloff, E.M., and Avruch, J., J. biol. Chem. *254* (1979) 245.
16 Assimacopoulos, F.D., Blackmore, P.F., and Exton, J.H., J. biol. Chem. *257* (1981) 3759.

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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