the experimental condition; for example, the main cation, SR concentration, and DEP concentration (not described in Shoshan-Barmatz's paper). However, no further study was attempted here.

The inhibition of Ca-ATPase by DEP was not affected by magnesium. Activity in 1 mM DEP relative to control was 0.97 ± 0.20 (n = 4) or 0.86 ± 0.13 (n = 5) in 1 mM or 3 mM $MgCl_2$, respectively. One mM DEP reduced the enzyme activity to 0.81 ± 0.02 of the control in the presence of 5 μM ruthenium red. These results indicate that inhibition of Ca-ATPase is not the direct cause of calcium release by DEP. It may be that DEP modifies calcium-releasing mechanisms, for example, the calcium channel itself or the receptor for signals from transverse tubules, and enhances the release of calcium from SR.

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Depressor effects of muscarinic and non-muscarinic mediation induced by lateral hypothalamic stimulation in the cat

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Summary. Transient sympathetically-mediated depressor effects were induced by stimulation of a small locus in the lateral hypothalamic peri-fornical region, medial to the fields of Forel. The ganglionic blocking agent, atropine methyl nitrate (ATMN), was used to show that muscarinic as well as non-muscarinic sympathetic ganglion receptor neurotransmission was involved. Evidence is presented that stimulation of this LH site co-activates a number of mechanisms and that depending on which of these are activated, the ganglionic blocking agent ATMN may either block, reverse or potentiate the depressor effect.
Key words. Hypothalamic-induced depressor effects; muscarinic-mediated BP; depressor effect blockage; depressor effect reversal; depressor effect potentiation.

Transient changes in blood pressure (BP) and heart rate (HR) normally take place in response to specific situational stimuli. These in turn activate antagonistic mechanisms that act to re-establish homeostatic conditions. However, disturbed autonomic patterns may persist due to failure of stabilizing mechanisms [7, 16, 17], possibly mechanisms other than the better known baroreceptor system [12].

A study in search of alternative control mechanisms was undertaken on an experimental model developed by us for the study of transient disturbances in BP and HR [3–5]. In this model, stimulation of specific sites in the lateral hypothalamus (LH) induces transient pressor and/or depressor effects, that are uniquely not associated with a change in HR [5].

In a previous study on a pressor effect induced by stimulation of an adjacent site, a sympathetic, nicotinic-receptor ganglionic mediation was revealed [4, 5]. We also found a tendency towards HR constancy or for small HR increases. Along with the BP rise, these were accounted for on the basis of baroreceptor suppression by such stimulation [11]. We have previously reported lack of vagal involvement [3]. Inhibitory mechanisms, presumably of the type described by Libet [14, 15] in sympathetic ganglia, were postulated to be involved in interactions with the above-mentioned neurotransmission mechanisms, to attenuate them. Their blockage was assumed to lead to the potentiation previously reported for the pressor effects, and observed in the present work for the depressor effects. Evidence was obtained for both muscarinic and

Table 1. Control mean values and SD of BP, PP and HR, for all the cats in the study

BP	170/115 ± 7/10
PP	60 ± 10
HR	140 ± 20

non-muscarinic ganglionic neurotransmission of depressor effects induced by stimulation of a site in the same region. These are also shown to be attenuated by a ganglionic, inhibitory muscarinic mechanism distinct from the baroreceptor mechanism.

The study was carried out on 17 cats of a Swiss breed grown in our animal house. In order to ensure randomicity of the cats used, means and SDs of control BP, pulse pressure (PP) and HR of the whole cat population of the study were evaluated as characteristic control values (table 1).

Experiments were performed in two stages a week apart, under α-choralose anesthesia (55–65 mg/kg dissolved in saline at 20 mg/ml). Arterial blood pressure was recorded from the femoral artery, using a venflow trochar connected through heparinized saline solution to a Statham pressure transducer. Blood pressure, and ECG in lead II, were recorded on a Grass polygraph.

Electrical stimulation of LH at stereotaxic coordinates[2] Fr:9.0, L:2.2–2.5, d: − 1 to − 2 was carried out by means of stainless steel wire electrodes. The type of stimulation electrodes, the techniques of chronic implantation, the stimulation parameters used and the technique for verification of electrode placement were as previously reported[3,10].

Ganglionic neurotransmission of the depressor effect was investigated with the peripherally acting muscarinic blocking agent atropine methyl nitrate (ATMN). In 17 cats ATMN was administered systemically (200 μg/kg), and in 10 of the same group of cats, at another stage of the experiment, ATMN (50 μg) was directly applied to the stellate ganglia. The administration of ATMN caused a transitory depressor effect of 27 ± 10 mm Hg in most and a small drop of PP and HR in some of the animals, with a return to baseline in all cases prior to the delivery of the LH stimulation.

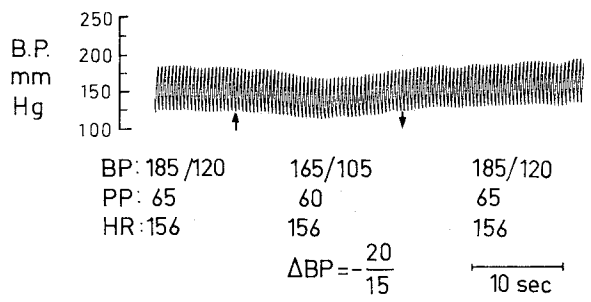

Figure 1. Illustrating a sample depressor effect, ΔBP = − (20/15 mm Hg), induced by stimulation of LH locus shown in figure 2. These depressor effects last throughout the stimulation, then returning gradually to base-line. Note minor changes in PP and, notably, the lack of change in HR.

In a previous study[5], control experiments pertinent to the present one were carried out, which negated the possibility that ATMN applied directly to the stellate ganglia leaked out to produce the effect at a site away from the point of direct application. We therefore studied in the first stage the effects of LH stimulation under action of ATMN administered systemically, 15 min prior to stimulation. In a second stage the stellate ganglia were microsurgically exposed and desheathed in situ, in order to study the effects of LH stimulation under action of ATMN applied directly to the stellate ganglia of one or of both sides.

The depressor effects studied (fig. 1) were obtained by stimulation within an area just medial to the fields of Forel (fig. 2). We included in the analysis only preparations in which the sites of stimulation were confirmed to be within this LH region. It is noteworthy that the BP

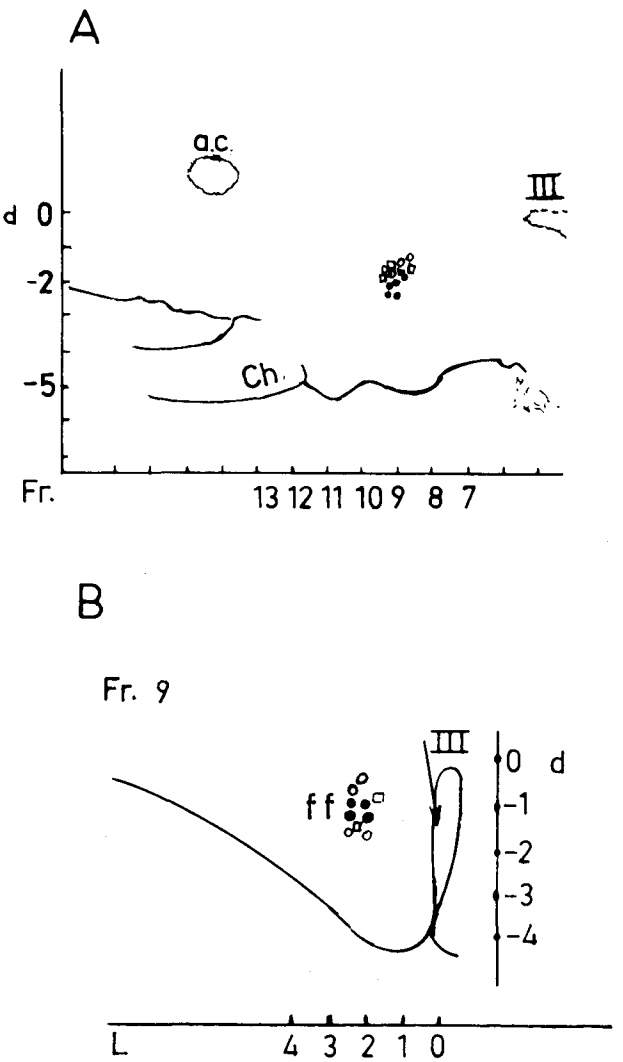

Figure 2. Illustrating anatomical data on the LH site of stimulation. In semi-schematic, sagittal (*A*) and frontal (*B*) sections loci are shown, the stimulation of which gives rise to depressor effects (o), adjacent to a pressor region (.) and to sites giving biphasic effects (□). Abbrevations: a.c., anterior commissure; Ch., chiasma; III, 3rd ventricle; Fr., Frontal; L, lateral; d, depth. coordinate; ff, fields of Forel.

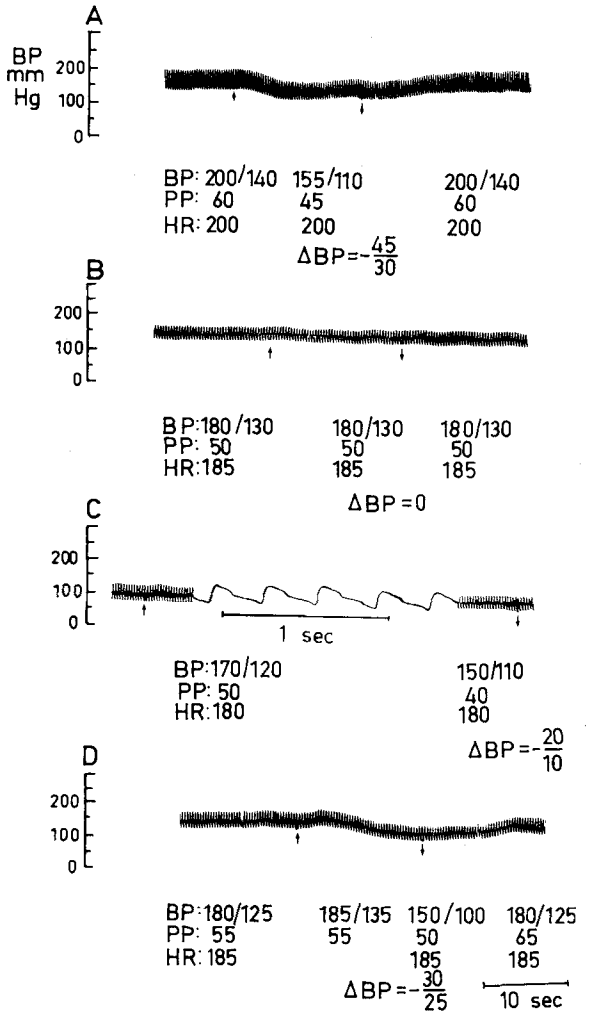

Figure 3. LH stimulation-induced depressor effect in another cat; blockage by systemic ATMN. *A* LH stimulation-induced depressor effect $\Delta BP = (-45/30)$ mm Hg, and a PP drop but no change in HR. *B* Complete blockage of the depressor effect by 200 µg/kg ATMN administered, i.v., 15 min earlier. $\Delta BP = 0$. *C* LH stimulation 44 min after ATMN induces a partial depressor effect. $\Delta BP = -(20/10)$, minor changes in PP, and no change in HR. *D* Further fading off of ATMN effect 166 min after its administration. This is indicated by LH stimulation inducing a $\Delta BP = -(30/25)$. No change in HR.

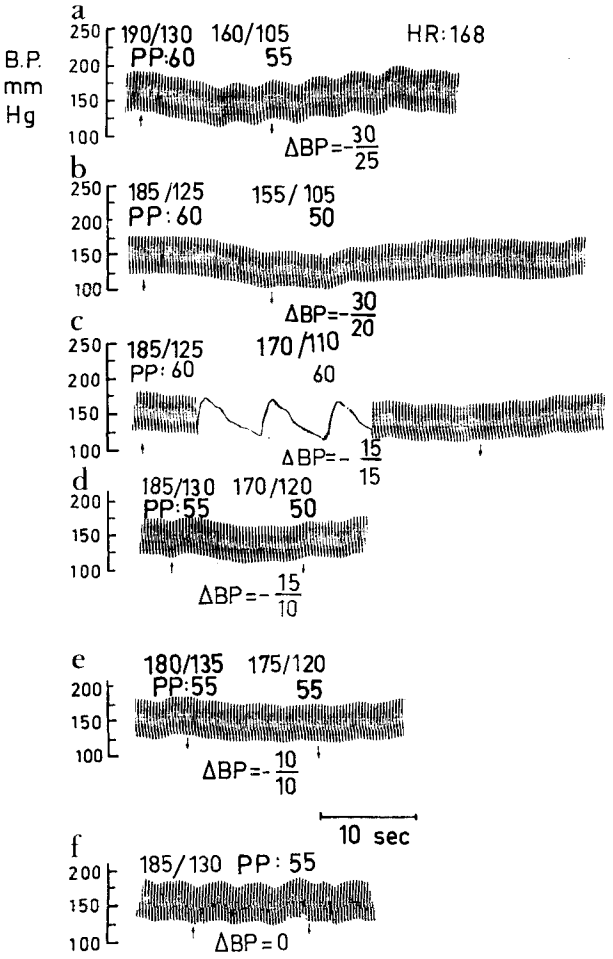

Figure 4. A blockage or the LH- induced depressor effect shown in the same cat by 50 µg ATMN applied directly to each of the desheathed stellate ganglia.
a) A depressor effect ($\Delta BP = -(30/35)$ caused by the LH stimulation.
b) 3 min after direct application of ATMN to the ganglia. $\Delta BP = -(30/20)$.
c) 7 min after direct application of ATMN to the ganglia, $\Delta BP = -(15/15)$. Partial blockage is already manifested.
d) 18 min after direct application of ATMN to the ganglia: $\Delta BP = -(15/10)$.
e) 48 min after direct application of ATMN to the ganglia: $\Delta BP = -(10/10)$.
f) 90 min after direct application of ATMN to the ganglia: $\Delta BP = 0$.

changes, though looking alike when measured systemically, were found differentiable on the basis of responses to ATMN treatment into three different types.

In one group of 9 cats (group 1) a depressor effect caused by LH stimulation was blocked completely in 8/9 cats, and reduced to almost 50% in 1/9 cats by systemic ATMN (fig. 3a–d). At another stage of the experiment ATMN applied directly to the ganglia was shown to block the depressor effect in 6 out of the 9 cats, the blockage simulating the systemic ATMN only acting at shorter latencies (fig. 4a–f). This effect suggested mediation of the depressor effect by a muscarinic ganglionic receptor. In a second group of 4 animals (group 2) the same systemic ATMN dosage, in two of which also direct application of 50 µg ATMN to the ganglia induced a reversal of the depressor effect (fig. 5). We assume that in these animals the LH stimulation co-activates a pressor and a depressor effect, with a net depressor effect. ATMN blocks the presumably muscarinic-mediate depressor component (as in group 1) and thus unmasks the pressor effect, and this is manifested here as a reversal phenomenon. This pressor effect was verified as an effect that is nicotinically mediated at the ganglion [5]. In a third group of 4 animals (group 3), the depressor effect induced seemed to be of non-muscarinic mediation. It was not suppressed but rather potentiated 1.5–4-fold by ATMN ganglionic blockage (fig. 6). In 2 of these animals the potentiation was obtained with application of

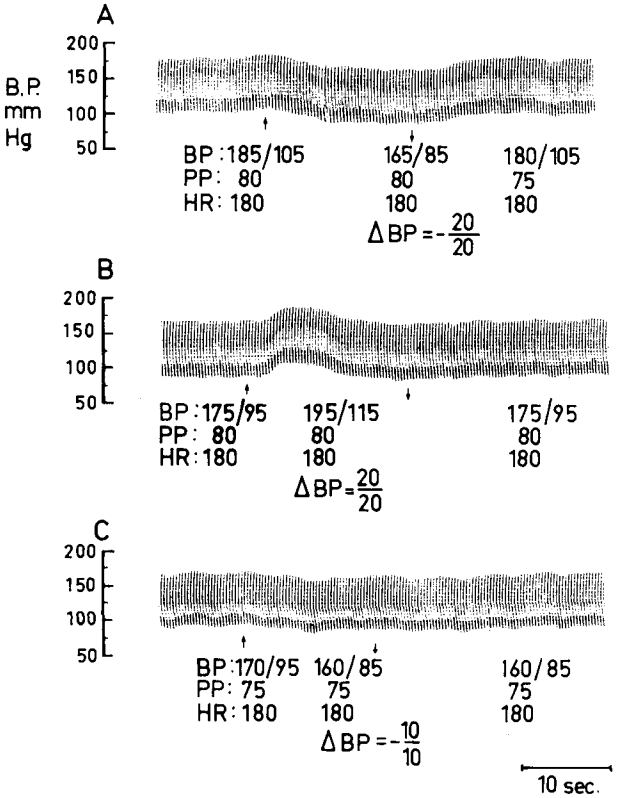

Figure 5. Illustrating ATMN (200 µg/kg i.v.) reversal effect on the depressor effect: *A* LH stimulation induces $\triangle BP = -(20/20)$ with no change in PP or in HR. *B* 21 min following ATMN LH stimulation induces a rise instead of a fall in BP. Note: $BP = +(20/20)$, no change in HR or PP. *C* After 80 min the reversal effect of ATMN is beginning to fade off; stimulation produces a small depressor effect $BP = -(10/10)$; No change in HR or PP.

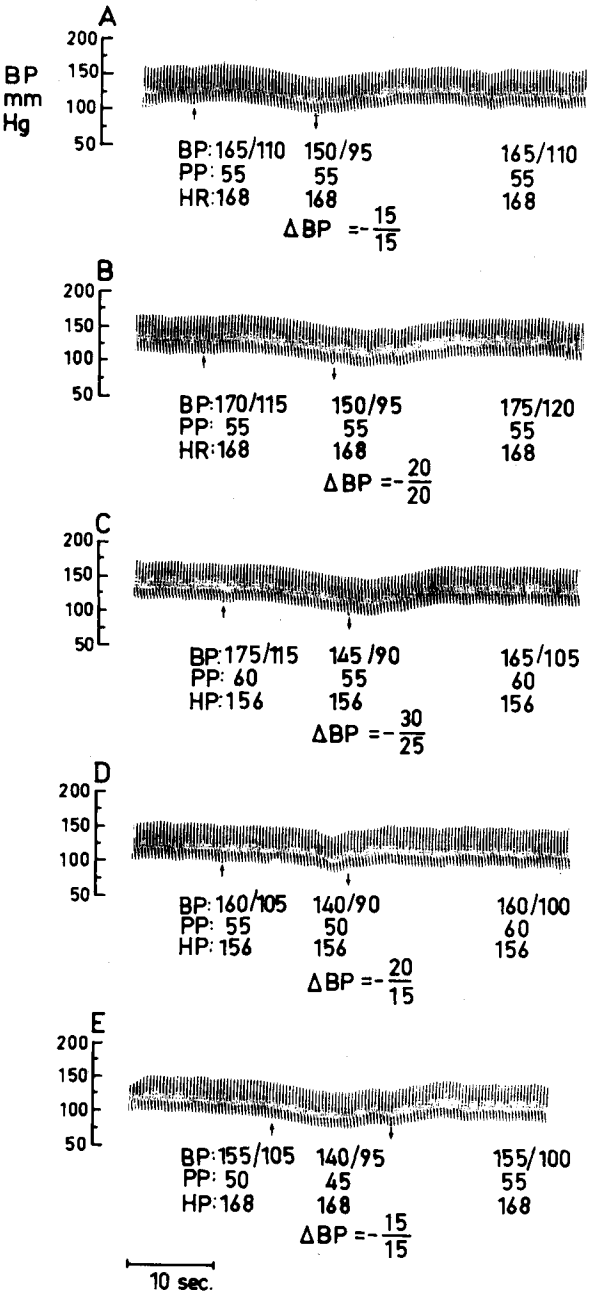

Figure 6. Illustrating the potentiation phenomenon caused by ATMN on the depressor effect. *A* Control. LH stimulation induces a depressor effect: $\triangle BP = -(15/15)$, with no change in PP or HR. *B* Potentiation of the depressor effect after 200 µg/kg i.v. ATMN. LH stimulation 7 min following ATMN: $\triangle BP = -(20/20)$ with no change in HR or PP. *C* The potentiation is increased at 53 min after drug administration: $\triangle BP = -(30/25)$. PP and HR are practically unchanged. *D* 80 min after ATMN administration, potentiation starts to decrease: $\triangle BP = -(20/15)$, and only minor PP or HR change. *E* 90 min after ATMN administration, potentiation has already faded off: $\triangle BP = -(15/15)$.

ATMN directly to the ganglia. It is noteworthy that this potentiation of the depressor effect bears a similarity to that which we have previously reported for the pressor effect [5]. We postulate for the depressor effect potentiation that it may also result from co-activation of a muscarinic sympatho-inhibitory mechanism [1] that in turn interacts with a non-muscarinic sympatho-excitatory mechanism, responsible for the depressor effect.

As the grouping described could be an arbitrary result of choice of cats in the study of the various aspects, control means and SDs of BP, PP and HR were evaluated for each of these groups as summarized in table 2.

Comparison of these with values in table 1 shows that they are in the same range, presumably derived without bias from the whole population.

We may, therefore, conclude that this grouping is due to activation of different pathways mediating different effects; pathways that are co-activated because they either originate or run in close proximity to one another in the LH, so that stimulation activates one or another mechanism or a combination of these.

It may be assumed that the depressor effects reported here are identical with those reported by Hess [10] for prac-

Table 2. Control means and SD of BP, PP and HR for each group

Group 1	Group 2	Group 3
BP 165/114 ± 2/14	175/105 ± 5/12	175/120 ± 9/5
PP 55 ± 5.5	70 ± 7	56 ± 6
HR 140 ± 20	155 ± 15	140 ± 15

tically the same hypothalamic region and in a similar animal species. However, we have gone a step further by accurately characterizing the effects with use of a ganglionic blocking agent, ATMN. This included verification of the ganglionic site of action by the application of the agent directly to the in situ, exposed ganglia. The dramatic effect that application of ATMN to the stellate ganglia has on the LH-induced depressor effect may be more easily understood in the light of some of our recent findings, that show (Blum et al., unpublished results) that the BP changes may be due to localized vascular effects. This may explain in part also the lack of change of heart rate. The results obtained enable us to differentiate the depressor phenomenon into a number of different types. As pointed out earlier, table 2 indicates that a population bias is not involved in this classification. It may be concluded that although activated practically from the same LH region, at least three different mechanisms are involved in the phenomena manifested. Figure 7 attempts to clarify this concept. It shows a number of parallel pathways, descending from LH and earmarked for control of different transients. Our evidence suggests that one of these is a muscarinic mediated ganglionic mechanism, equatable perhaps with the one described in sympathetic ganglia by Flacke and Gillis[8]. The latter authors identified this mechanism by stimulation of pre-ganglionic fibers entering the stellate ganglion. It is noteworthy that they stimulated the total nerve bundle whereas in our case, stimulation was limited to the activation, sometimes, of just the depressor pathway by itself. In addition, here, the hypothalamic source of the phenomena is specified.

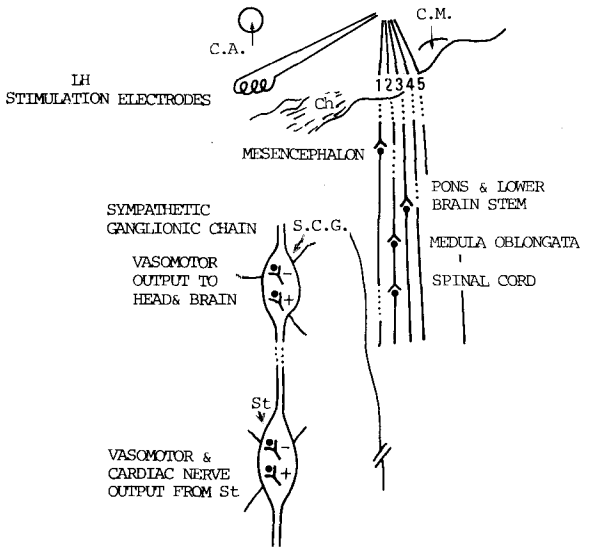

Figure 7. A schematic illustration of the parallel pathways (1, 2, 3, 4, 5..) descending from lateral hypothalamus (LH), of sites of implanted stimulation electrodes and of landmarks: C.A., commissura anterior; C.M., corpora mammillary; Ch, optic chiasma. These pathways form synapses at mesencephalic, pontine and lower brain stem, medulla oblongata and spinal cord way-stations as previously reported[6,9]. We show here sympatho-excitatory (+) and sympatho-inhibitory (−) synapses at S.C.G. = superior cervical ganglion and St = stellate ganglia.

The above-mentioned mechanism is offered also as an explanation of the observed reversal phenomenon which we assume to result from the simultaneous activation of a pressor and a depressor effect with a net depressor effect; ATMN suppression of the muscarinic component unmasks the pressor effect. Another phenomenon observed under ATMN ganglionic blockage was a depressor effect potentiation. This phenomenon reflects a non-muscarinic mechanism, confirmed by us in some animals (Blum et al., unpublished) to be nicotinic. This non-muscarinic depressor effect is not suppressed but rather potentiated by systemic ATMN administration. The same effect has been observed when the drug was applied directly to the ganglia, although at shorter latency.

We suggest that this potentiation of the depressor effect is similar to that which we have previously reported for the pressor effect[5]. We assume for either of these that inhibitory effects are co-activated by the LH stimulation along with the depressor or the pressor effects. We assume that this hypothesized muscarinic sympatho-inhibitory mechanism[5] interacts at ganglionic synapses with the non-muscarinic sympatho-excitatory depressor or pressor effect. We thus extend our previously offered hypothesis about the potentiation phenomenon[5] to include the depressor effect, i.e. that blockage by ATMN of interactions of sympatho-inhibitory pathways[1] with depressor effect mediating pathways are the basis also of the depressor effect potentiation reported here. We believe that the sympatho-inhibitory pathway[6,9] involved is distinctly different from the sympatho-inhibitory pathways of the baroreceptor system[1,13], as it is activated when the baroreceptor mechanism is under suppression. The depressor effect was in some cases completely suppressed by bilateral application of ATMN just to the stellate ganglia. This concurs with a rather localized vascular effect of the LH stimulation. It seems that interactions at the ganglionic level between sympatho-excitatory depressor pathways and a distinct sympatho-inhibitory mechanism result in attenuation of the depressor effect, and the blockage of these leads to the manifestation of depressor effect potentiation. It is proposed that the mechanism revealed is responsible for protection against excessive lowering of blood pressure, in a similar way to the protection against excessive rises in blood pressure shown by us previously[5].

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Further characterization of sulfated homopolysaccharides as anti-HIV agents

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Summary. Fucoidan and dextran sulfate showed anti-HIV activities against mononuclear cells from AIDS patients, and they abrogated HIV reverse transcriptase (RT) activity by interacting with the HIV envelope in the membranes of target cells. Furthermore, they showed a synergistic effect with azidothymidine (AZT).
Key words. Dextran sulfate; fucoidan; anti-HIV agents.

It is important and urgent to find a potent anti-HIV agent that can be utilized safely under clinical conditions. We have previously reported that sulfated homopolysaccharides with immunomodulating activities are more potent anti-HIV agents than sulfated heteropolysaccharides[1]. This study evaluates the actions of dextran sulfate and fucoidan, two typical sulfated homopolysaccharides, against peripheral mononuclear cells (MNC) from two AIDS patients in vitro, and attempts to elucidate the mechanism of action of these chemicals.

Materials and methods

Peripheral MNC from two hemophiliacs with AIDS (cases 1 and 2) were obtained by centrifugation over Ficoll-Paque cushions at 2000 rpm for 20 min[2]. The MNC were co-cultured for 10 days with lymphoblasts activated with PHA (10 μg/ml) for three days in the presence of dextran sulfate or DEAE dextran as a negative control. Reverse transcriptase (RT) activities in the culture supernatants were measured according to the method of Daniel et al.[3]. Briefly, Molt-4. clone no. 8 cells were cultured with the supernatant from HIV-infected TALL-1 cells. The cell suspension was centrifuged at 1300 rpm for 10 min. The supernatant (1.5 ml) was centrifuged at 25,000 rpm for 90 min to pellet viruses. The virus pellet was resuspended in 10 μl of dissociation buffer. To this was added 90 μl of a solution containing 0.04 M Tris-HCl, 0.1 M $MgCl_2$, 0.045 M KCl, poly (rA): oligo (dT)/ml, and 0.004 M dithiothreitol, and then 2.5 μl of [methyl-^{3}H]thymidine 5'-triphosphate (30 Ci/nmol) was added to the reaction mixture. After shaking in a water bath at 37 °C for 60 min, each sample (100 μl) was spotted onto a glass fiber filter, washed, and rinsed with ethanol. The filter was counted in a liquid scintillation counter. The background count (HIV-free) was usually less than 2500 cpm.

Next, the following experiments were carried out to elucidate the mechanism of action of dextran sulfate and fucoidan. Molt-4. clone no. 8 cells (10^6/ml) as a target[4] were cultured overnight at 37 °C in a humidified incubator with 5% CO_2 in air in the presence of fucoidan (100 μg/ml) or dextran sulfate (100 μg/ml). Thereafter, the target cells were carefully washed three times and were cultured for 10 days with HIV-containing supernatants whose RT activity was more than 5×10^5 cpm/ml. The effect of fucoidan or dextran sulfate upon HIV was evaluated in terms of RT activity and cytopathic affect (CPE) score[5].

Furthermore, in order to clarify the mechanism of action in more detail, Molt-4. clone no. 8 cells were cultured in the presence of HIV for four different time intervals (2, 6, 12, and 24 h). Thereafter, the cells were carefully washed three times so that the remaining HIV were removed completely. The HIV-treated cells were cultured in the presence of fucoidan and dextran sulfate for 10 days and RT activities in the culture supernatants, as well as the CPE score, were determined as described above. A