Action and interaction of two α_1-adrenoceptor agonists, methoxamine and St 587, on the rabbit's blood pressure and EEG

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Summary. In rabbits methoxamine reversed the vasopressor effect of St 587 and abolished the EEG synchronizing action of St 587. The interaction on the blood pressure could be ascribed either to the different chemical structures of St 587 and methoxamine or to partial agonistic properties of St 587. The interaction on the EEG appears to be more complex.
Key words. Methoxamine; St 587; blood pressure; EEG.

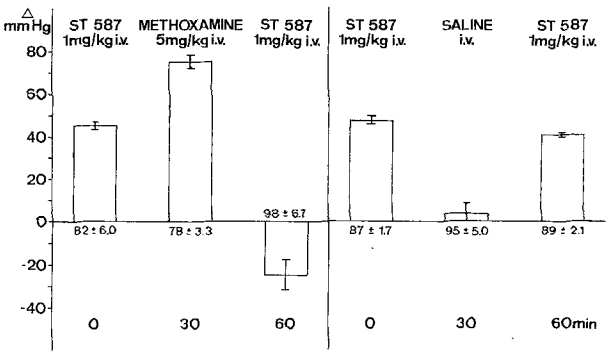
Methoxamine and ST 587 are widely accepted as highly selective prototype agonists at α_1-adrenoceptors [1, 2]. St 587 has been characterized as a very lipoid-soluble drug [3]. Methoxamine has also been found to penetrate the central nervous system easily [4], and its hypertensive effect has been ascribed to both peripheral and central actions [5]. Consequently, central effects can be expected from both drugs. Recently we described a biphasic effect of St 587 on the rabbit's EEG, an EEG synchronization preceded by a short period of cortical arousal [6]. Because we were interested in the question of whether these effects are specific for St 587 or for central effective α_1-adrenoceptor agonists in general, we wanted to compare the effects of St 587 and methoxamine on the rabbit's EEG and blood pressure in the same animal, and on this occasion we noticed interesting interactions between these two drugs.

Materials and methods. Eighteen rabbits of either sex (2.3–3.3 kg) were used in this study. For recording the blood pressure a polyethylene catheter was inserted through an incision into the central ear artery after local anesthesia of the base of the ear with 1 ml of a 2% procaine hydrochloride solution. The mean arterial blood pressures were 90–100 mm Hg during the control periods. The method of Monnier and Gangloff [7] was employed for EEG recording because it allows experimentation in conscious, noncurarized animals without any pain or discomfort. The EEG was recorded from the precentral, parietal and occipital cortex. During the control period, as well as after the administration of St 587 or methoxamine, proprioceptive stimulations (passive flexion and extension of a hind limb) were carried out repeatedly. Aqueous solutions of methoxamine and St 587 (2-(2-chloro-5-trifluoromethylphenylimino)imidazoline nitrate) were freshly prepared for each experiment and administered intravenously. After the experiment the animals were sacrificed with an overdose of pentobarbital sodium.

Results. 1) Actions on blood pressure and EEG of St 587 and methoxamine, given alone. St 587, in a dose range of 0.125 to 8 mg/kg, causes a dose-dependent increase of the mean arterial blood pressure and a biphasic EEG effect, an EEG synchronization preceded by a short period of cortical arousal. Details of these actions have been reported recently [6].

Methoxamine was given in single doses (1, 2 and 5 mg/kg) in three experiments and in cumulative doses (starting with 0.125 mg/kg and increasing the doses by a factor of two every 10 min up to a total dose of 8 mg/kg) in five experiments.

The single doses increased the mean arterial pressure by 70, 80 and 85 mm Hg, respectively. In the experiments with cumulative doses there was a progressively more pronounced and longer-lasting hypertensive effect as the total dose was increased, up to maximal values of about 200 mm Hg after total doses of 8 mg/kg. Immediately after the injection of methoxamine in doses of 1 mg/kg or more an EEG desynchronization occurred concomitantly with the initial increase in blood pressure. After the highest doses used in this study (total doses of 8 mg/kg), periods of cortical desynchronization lasting up to several minutes occurred in association with movements of the animals. After doses of 1 mg/kg or less (in the cumulative doses series), there was an attenuation and/or abbreviation of the cortical arousal reaction induced by proprioceptive stimulations, lasting up to 5 min after the injection.



Maximal change of mean arterial pressure (in mm Hg, ordinate, ±SEM; values at ordinate are basal values immediately before drug injection) in two groups of three animals each after the treatments as indicated in the heading. Note reversal of St 587-induced hypertensive effect after methoxamine.

2) Interaction on blood pressure and EEG of St 587 and methoxamine. In six rabbits the interaction on blood pressure of St 587 and methoxamine was studied. These animals were given 1 mg/kg St 587 twice at an interval of 60 min. Three animals received 5 mg/kg methoxamine 30 min after the first St 587 injection, the other three animals received saline instead of methoxamine and served as controls. The figure shows the results. Both methoxamine and St 587 elevated the blood pressure. As indicated by the basal values, this hypertensive effect was of short duration (<30 min) after St 587, but of long duration after methoxamine (>30 min). Furthermore, as the figure shows, the cardiovascular effect of St 587 was reversed into hypotension when given 30 min after methoxamine. In four EEG experiments with the same dosage schedule it was found that methoxamine also changes the central actions of St 587: the EEG synchronization of St 587 was abolished while the initial EEG desynchronizing effect of St 587 remained unchanged by methoxamine.

Discussion. In this study, using two selective α_1-adrenoceptor agonists (see above), methoxamine caused a reversal of the vasopressor effect of St 587, a finding which came completely unexpected and is not easy to explain. Such an interaction requires both the presence of methoxamine on the receptors at the time when St 587 was given (i.e., 30 min after injection) and the existence of differences in the modes of action of these two drugs.

Methoxamine has been described as a drug with a long duration of action [4], and in the present interaction study the total peripheral resistance was still elevated 30 min after the injec-

tion. As to differences in the modes of action, there appear to be at least two possibilities. First, they could be due to the different chemical structure of methoxamine and St 587: whereas methoxamine contains a phenethylamine structure, St 587 contains an imidazoline ring. Phenethylamines and imidazolines were assumed to interact differently with α-adrenergic receptors[8]. It was found later that St 587 (like clonidine) has a hypotensive effect when injected into the nucleus reticularis lateralis region, in contrast to catecholamines like norepinephrine or the α_2-adrenoceptor agonist α-methylnorepinephrine. Consequently, imidazoline-preferring sites were postulated to exist in this region[9]. Recent findings suggest, moreover, that these imidazoline binding sites are neither adrenoceptors nor histamine receptors[10]. It is thus likely that St 587, being an imidazoline derivative, has an affinity to both imidazoline binding sites and α_1-adrenoceptors. Methoxamine would unmask the hypotensive action of St 587 at the imidazoline binding site. An alternative explanation is provided by the fact that St 587 proved to be a partial agonist under certain experimental conditions (i.e. on the isolated rat hindquarter preparation[11]). A partial α_1-adrenoceptor agonist, given after methoxamine, would be expected to act as an antagonist rather than an agonist. This would also result in a decrease in blood pressure.

An EEG desynchronization induced by methoxamine has also been reported by other authors, and it was assumed that this stimulant effect is independent of the blood pressure increase[12]. We have reported previously[6] that St 587 induces a biphasic EEG effect, and EEG synchronization preceded by a short period of cortical arousal. The present study confirmed these results and showed, moreover, that the St 587-induced EEG synchronization is abolished by methoxamine. It is difficult to explain this interaction on the same basis as the interaction on the blood pressure. The reported data would indicate that 1) the initial EEG desynchronization induced by St 587 is not caused by its hypertensive effect and 2) the two phases of the St 587-induced EEG changes cannot be caused by a single, common effect of this drug on one receptor (sub)type. Certainly, this phenomenon deserves further experimentation with other pairs of α_1-adrenoceptor ligands.

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Choline accumulation in isolated rat hepatocytes

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Summary. The accumulation of non-metabolized choline in isolated rat hepatocytes is concentrative in Na^+ medium, whereas the accumulation does not exceed unity in a Li^+ medium. Ouabain and 2,4-dinitrophenol inhibited the choline uptake. These results indicate that choline is taken up by rat hepatocytes via a Na^+- and energy-dependent process, and choline oxidase is not directly connected with the choline transport system.
Key words. Choline; choline accumulation; Na^+- and energy-dependent process; choline analogs; quaternary ammonium compounds; rat hepatocytes.

Choline, in the form of the phospholipid phosphatidylcholine (lecithin), is an integral component of all membranes and has various important physiological roles; among others, as methyl donor for acetylcholine synthesis, and as a lipotrophic factor. The transport of choline has been studied in various different tissues such as red blood cells[1,2], brain[3-5], hepatoma cells[6], diaphragm[7], kidney[8-11], small intestine[12] and liver[13-15]. In red blood cells[1,2], brain[4,5], diaphragm[17] and kidney[8] it has been demonstrated that choline uptake may occur against a concentration gradient. Hepatic choline transport has been studied by some investigators in perfused liver and in isolated rat hepatocytes. In the study using perfused rat liver Tuma et al.[13] suggested that choline uptake by the liver is directly connected with choline oxidase. On the other hand, Zeisel et al.[14] showed that non-metabolized choline was accumulated within the intracellular space, suggesting that choline oxidase does not necessarily limit choline uptake in the liver. Our previous paper[15] demonstrated that choline was transported in isolated rat hepatocytes by two mechanisms; one is a saturable mechnaism with a K_t of 162 ± 3.85 μM and V_{max} of 80.1 $\pm$ 1.30 pmol/10^5 cells per min, the other is a non-saturable mechanism. However, it is still unclear whether the choline transport is directly related to its metabolism. Therefore, in this study, we further characterized choline transport in isolated rat hepatocytes. We demonstrated that choline is taken up by an Na^+- and energy-dependent process and that quaternary ammonium compounds which have a negatively charged group in the molecule do not show any inhibitory effect on choline uptake.

Materials and methods. [^{14}C]Choline ([methyl-^{14}C]choline chloride, 58 Ci/mol) was purchased from Amersham International (U.K.). The following agents were used: collagenase (CLS IV) from Worthington, bovine serum albumin