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

(Fraction V powder) from Miles and Amberlite CG50 (type 2) from Rohm & Haase Company. All other chemicals were of reagent grade.

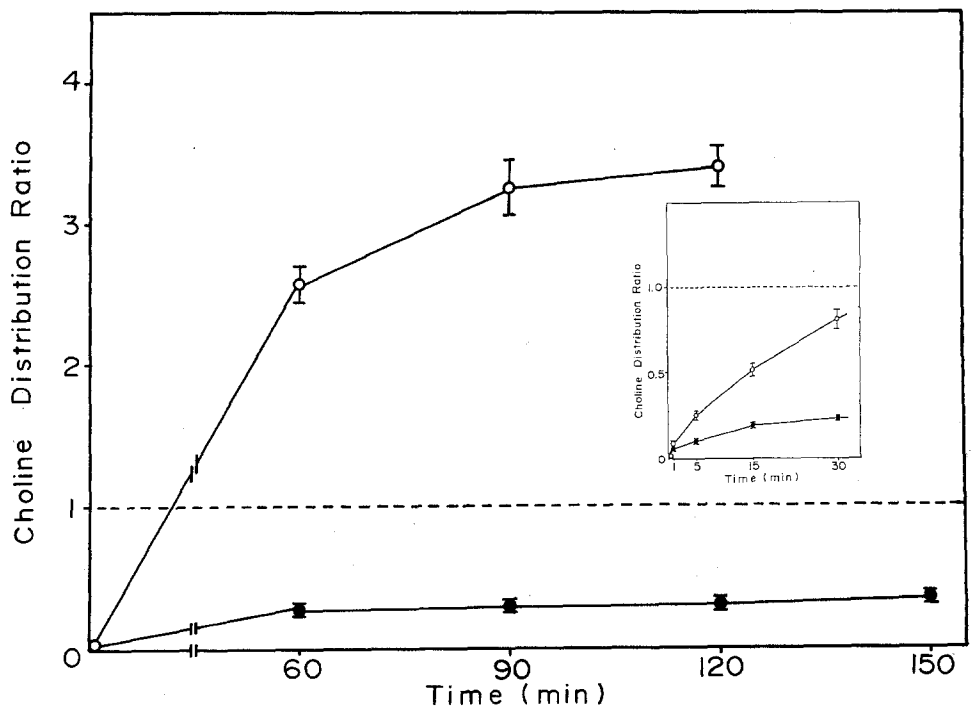
Hepatocytes were prepared from 200–300 g Wistar male rats fed ad libitum as previously reported [15]. After preincubation for 15 min at 37 °C, the transport of choline was initiated in a Corning centrifugation tube (50 ml) by the addition of [^{14}C]choline to 3 ml of cells suspended (3.5×10^6 cells/ml) in the Krebs-Henseleit medium containing dialyzed bovine serum albumin (25 mg/ml), streptomycin (100 ng/ml), penicillin G (100 units/ml). Uptake was terminated by the addition of 15 ml of ice-cold medium. After separation of the medium from the cell pellets by centrifugation for 5 s at $700 \times g$, the cell pellets were washed with 10 ml of ice-cold medium, and then recentrifuged for 5 s at $700 \times g$ as described previously [15].

In the experiments on the effect of Na^+ depletion on the uptake of choline, the cells were centrifuged at $50 \times g$ for 15 s after preincubation for 15 min and then resuspended in the same volume of a Li^+ medium in which LiCl was substituted for NaCl and $KHCO_3$ for $NaHCO_3$. After the cells in the Li^+ medium had been centrifuged again at $50 \times g$ for 15 s and resuspended in the same volume of the Li^+ medium, the transport experiments were initiated by the addition of [^{14}C]choline at the concentrations indicated. The control experiments were carried out under the same conditions. The preincubation and incubation were carried out at 37 °C and the mixtures were equilibrated with 95% O_2 and 5% CO_2 at all times. Blank tubes were routinely determined as follows; [^{14}C]choline was added to the cell suspensions at 0 °C, and the suspensions immediately diluted, centrifuged and washed by the procedure described above. In the experiment measuring total choline uptake, cell pellets were extracted by the addition of 1 ml of 6.3% trichloroacetic acid and the radioactivity was measured in a liquid scintillant containing Triton X-100, by means of liquid scintillation spectrometry. Labeled non-metabolized choline in the cells was separated as follows: after the uptake of [^{14}C]choline into isolated rat hepatocytes, cell pellets were homogenized in 2 ml of 1% solution of Triton X-100 and the homogenates were loaded onto a 5×0.6 cm column of Amberlite CG50 resin in the sodium form. After elution of choline metabolites with 10 ml of water, the non-metabolized [^{14}C]choline was eluted with 10 ml of 0.1 N HCl [19]. The compound eluted from the column with 0.1 N HCl was identified as choline by paper electrophoresis according to the procedure of Potter and Murphy [20].

The intracellular water space was determined as the difference of the ^3H_2O and [^{14}C]inulin distribution ratios in the cell pellets [18] in parallel for each experiment, and calculated to be 2.63 ± 0.222 µl/10^6 cells (n = 45, mean ±SE). Cell viability was determined by the Trypan blue exclusion method.

The data for choline transport obtained are presented as mean ±SE. Kinetic parameters (K_t and V_{max}) and SE for these values were calculated as described by Wilkinson [21]. Significant differences were assessed by Student's two-tailed t-test.

Results and discussion. The previous paper from our laboratory [15] showed that choline uptake by isolated rat hepatocytes proceeded linearly with time during 5 min of incubation at 37 °C in a Na^+ medium, and the rate of uptake gradually slowed down thereafter. In this study, the intracellular accumulation of non-metabolized [^{14}C]choline in isolated rat hepatocytes was determined by separation from its metabolites with Amberlite CG50 resin in the sodium form. In a Na^+ medium containing an initial concentration of 50 µM, choline taken up by isolated rat hepatocytes was at first rapidly metabolized at 37 °C (96% of choline taken up in 1 min of incubation was metabolized). However, the accu-



Time course of non-metabolized [^{14}C]choline accumulation in a Na^+ medium and a Li^+ medium. Hepatocytes were incubated at 37 °C in a Na^+ medium (–○–) and in a Li^+ medium (–●–) containing an initial concentration of 50 µM [^{14}C]choline. Non-metabolized choline in hepatocytes was measured using Amberlite CG50 resin in the sodium form. Cell viability after 150 min of incubation remained above 80%. The distribution ratio is the molar ratio of the intracellular choline to choline in the medium. The results presented are the mean ±SE of three experiments.

mulation of non-metabolized [^{14}C]choline gradually increased with time and exceeded unity after incubation for 60 min (fig.). Moreover, at a near steady state 90–120 min the extra-/intracellular distribution ratio reached 3.4 (fig.). On the other hand, the intracellular accumulation of non-metabolized [^{14}C]choline in a Li^+ medium, in which LiCl was substituted for NaCl and $KHCO_3$ for $NaHCO_3$, was very slow and did not exceed unity even after incubation for 150 min (fig.). These results indicated that choline accumulation in isolated rat hepatocytes is an uphill process which is dependent on Na^+ in the medium.

The previous paper[15] also indicated that choline was taken up by two mechnisms; a saturable one with a K_t of 162 $\pm$ 3.85 µM, and a nonsaturable one. In order to investigate the effect of Na^+ in the medium on hepatocytic choline uptake, initial choline uptake (after incubation for 1 min) in a Li^+ medium containing 50 µM of [^{14}C]choline was compared to that in a Na^+ medium with the same choline concentration. The initial choline uptake in the Li^+ medium was reduced to 73% of that in the Na^+ medium. Furthermore, the initial uptake of [^{14}C]choline over a concentration range from 20 to 200 µM was measured both in the Na^+ medium and in the Li^+ medium. In the Li^+ medium, the K_t value for choline increased from 133.5 $\pm$ 8.03 µM to 187.3 $\pm$ 7.28 µM ($p < 0.001$) without affecting the V_{max} value (56.1 $\pm$ 2.16 pmol/10^5 cells per min in the Na^+ medium and 52.58 $\pm$ 1.14 pmol/10^5 cells per min in the Li^+ medium) (data not shown). These results suggested that Na^+ increases the affinity of the uptake system for choline in isolated rat hepatocytes.

Table 1 shows the effect of ouabain and 2,4-dinitrophenol on the initial rate of choline uptake by rat hepatocytes. The uptake of choline was inhibited 20% by 0.5 mM ouabain and 23% by 1 mM 2,4-dinitrophenol. These results suggest that choline uptake is a Na^+- and energy-dependent process. They also indicate that choline oxidase is not directly connected with choline uptake as described by Zeisel et al.[14].

The effect of some choline analogs at the concentration of 0.5 mM on the initial rate of [^{14}C]choline uptake in isolated rat hepatocytes is presented in table 2. Among the compounds examined, *N,N*-dimethylethanolamine and hemicholinium-3 were the most potent inhibitors of choline uptake. The potency was the same as that in chick small intestine as reported by Herzberg and Lerner[12]. These compounds inhibited choline uptake in rat hepatocytes 72% and 87%, respectively. Acetylcholine, tetraethylammonium, decamethonium and curare inhibited to the extent of 29, 23, 32 and 47%, respectively. Betaine and carnitine, which have a carboxyl group in the molecule, did not inhibit the choline uptake as described for chick small intestine[12]. Furthermore, phosphorylcholine had no inhibitory effect, like thiamine phosphates; thiamine monophosphate and thiamine pyrophosphate were previously shown not to inhibit choline uptake in isolated rat hepatocytes[15]. These results indicate that choline uptake in rat hepatocytes is not inhibited by quaternary ammonium compounds which have a negatively charged group in the molecule[16].

Since choline oxidase does not always limit choline uptake by rat liver, this uptake system is of interest in connection with its metabolism in liver and should be investigated further. We have already presented the interaction of thiamine uptake with choline uptake in isolated rat hepatocytes and suggested that thiamine and choline do not share a common transport site, on the basis of kinetic studies[15]. Furthermore, in order to elucidate their exact relationship, investigation at the molecular level of both thiamine and choline transport systems in rat liver may be necessary.

Table 1. Effect of ouabain and 2,4-dinitrophenol on choline uptake in isolated rat hepatocytes

Addition		[^{14}C]Choline uptake (pmol/10^5 cells per min)	Distribution ratio	Percent
None		16.29 $\pm$ 0.856	5.191 $\pm$ 0.273	100
Ouabain	0.5 mM	12.73 $\pm$ 0.955	4.057 $\pm$ 0.373 [a]	80
2,4-Dinitrophenol	0.5 mM	14.25 $\pm$ 0.184	4.544 $\pm$ 0.072 [a]	90
	1 mM	12.21 $\pm$ 0.146	3.891 $\pm$ 0.057 [b]	77

The uptake of 50 µM [^{14}C]choline for 1 min of incubation was assayed as described in the text, after incubation with ouabain or 2,4-dinitrophenol for 15 min. Cell viability remained about 80–90%. The data presented are corrected for the contribution of the nonsaturable component. The distribution ratio is the ratio of the amount of intracellular choline taken up to 50 µM choline in the medium. The results presented are the means $\pm$SE of three experiments. [a] Significantly different ($p < 0.05$) from none. [b] Significantly different ($p < 0.01$) from none.

Table 2. Effect of choline analogs on choline uptake in isolated rat hepatocytes

Addition	[^{14}C]Choline uptake (pmol/10^5 cells per min)	Distribution ratio	Percent
None	16.229 $\pm$ 0.594	7.637 $\pm$ 0.280	100
Choline	7.918 $\pm$ 0.169	3.726 $\pm$ 0.079 [a]	49
Phosphorylcholine	16.371 $\pm$ 0.234	7.704 $\pm$ 0.110 [c]	101
Betaine	16.366 $\pm$ 0.744	7.701 $\pm$ 0.350 [c]	101
Carnitine	16.300 $\pm$ 0.503	7.670 $\pm$ 0.237 [c]	100
Acetylcholine	11.525 $\pm$ 0.605	5.423 $\pm$ 0.284 [a]	71
Tetramethylammonium	14.683 $\pm$ 0.208	6.910 $\pm$ 0.098 [b]	90
Tetraethylammonium	12.561 $\pm$ 0.787	5.911 $\pm$ 0.371 [a]	77
Hexamethonium	15.613 $\pm$ 0.378	7.347 $\pm$ 0.178 [c]	96
Decamethonium	10.970 $\pm$ 0.951	5.163 $\pm$ 0.448 [a]	68
Curare	8.592 $\pm$ 0.472	4.043 $\pm$ 0.222 [a]	53
N,N-Dimethylethanolamine	6.105 $\pm$ 0.458	2.873 $\pm$ 0.216 [a]	38
Hemicholinium-3	2.154 $\pm$ 0.217	1.014 $\pm$ 0.102 [a]	13

The uptake of [^{14}C]choline was assayed as described in the text. Choline analogs at the concentration 0.5 mM were added to the liver cell suspensions simultaneously with 50 µM [^{14}C]choline and the mixtures were incubated for 1 min. The data are corrected for the contribution of the nonsaturable component. The distribution ratio is the ratio of the amount of intracellular choline taken up to 50 µM [^{14}C]choline in the medium. The results presented are the means $\pm$SE of three experiments. Cell viablity remained about 80–90%. [a] Significantly different ($p < 0.001$) from none. [b] Significantly different ($p < 0.02$) from none. [c] Not significantly different from none.

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In vitro enhancement of human platelet aggregation by somatostatin

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Summary. Very low concentrations of somatostatin (S-14) strongly potentiate the in vitro aggregation induced by collagen, ristocetin and arachidonic acid, but not that induced by ADP or epinephrine, in both human platelet rich plasmas and gel-filtered platelet preparations. Desensitization phenomena may be induced either by repeated addition of S-14 or long lasting contact between S-14 and platelets.
Key words. Somatostatin; platelet aggregation; platelet activation.

The involvement of somatostatin in hemostasis and blood clotting has been extensively investigated [1-7], but its role in these physiological mechanisms is still largely obscure. While clotting factors appear to be unaffected by somatostatin both in animals and humans, thrombocytopenia has been reported in chronically somatostatin-treated baboons [8] and 24 h after somatostatin infusion in man [5,6], in spite of a lack of changes in platelet survival [8]. Inhibition of platelet aggregation after in vivo infusion in man and rabbits has been reported by some investigators [8-10]; however, no significant change has been reported under the same conditions in both platelet adhesion and aggregation [11]. The production of circulating platelet aggregates was enhanced after infusion of somatostatin in diabetic, but not in normal subjects [12]. In vitro studies also led to conflicting results as regards the effects of somatostatin on platelet function: evidence has been provided of a marked inhibition of platelet aggregation induced by ADP, collagen and ristocetin, in the presence of very high concentrations of somatostatin [8], whereas increased responses to epinephrine have been reported by others [9,12].

Some information has been obtained in recent years about platelet receptors for polypeptide hormones such as vasopressin [13-17]; specific recognition sites for somatostatin have been identified in several cell types, but not in human platelets [18]. Nevertheless, in this communication evidence will be given of a potentiating effect of very low concentrations of S-14 on the in vitro human platelet aggregation selectively induced by some platelet agonists.

Samples of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were obtained from healthy aspirin-free students of the Rome University Medical School. In some experiments we used, instead of PRP, suspensions of plasmaprotein-free platelets, obtained by gel filtration according to Tangen and Berman [19]: these gel-filtered platelets (GFP) showed normal ultrastructure, with uniform distribution of granules and absence of pseudopodia or other signs of activation. These GFPs, resuspended in phosphate buffer, aggregated in response to thrombin (> 0.05 U/ml), arachidonic acid (> 50 μM) and ristocetin (> 1.5 mg/ml) also in absence of added human fibrinogen and Ca^{2+}; to elicit a response to ADP (> 0.8 μM), epinephrine (> 5 μM) and collagen (> 4 μg/ml), the addition of human fibrinogen (final concentration 1.5 mg/ml) and Ca^{2+} (final concentration 25 μg/ml) were required.

The aggregation assays were performed in plastic cells, using a Dual Channel Elvi-Logos 840 Aggregometer. ADP, epinephrine, collagen, ristocetin and arachidonic acid were used as inducers. For each specimen of PRP or GFP, we sought the concentration of each of the aggregating agents which was able to induce a minimal primary aggregation wave (threshold concentration): then concentrations as low as at least 1/5 of the threshold ones were added to PRP or GFP. Prior to the addition of each aggregation inducer, somatostatin was introduced in one of the two cells; its final concentration was in different experiments 250 pg/ml, 1 ng/ml, 60 ng/ml, 300 ng/ml, 1 μg/ml. The second cell was used as a control. The interval between addition of somatostatin and that of the aggregation inducer ranged from 5 s to 20 min. In some experiments a second addition of the same dose of somatostatin followed the first one in the same cell containing PRP or GFP, 20 min or more later.

Somatostatin alone did not induce platelet aggregation, either in PRP or in GFP. After addition of somatostatin to PRP, subthreshold concentrations of collagen (from 0.1 to 2 μg/ml), arachidonic acid (from 0.55 to 1.1 μM) and ristocetin (from 0.5 to 1 mg/ml) induced a nearly maximal aggregation response (fig. 1). The table analytically reports the experiments performed at a concentration of 60 ng/ml (see also figs 1 and 2); however, S-14 showed similar enhancing