and also enters the intact nerve terminals provides support for a dual mode of action, involving both binding to receptor sites and re-uptake inhibition of different neurotransmitter systems.

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Dissociation constants of 4-acetoxy-piperidines and -thiacyclohexanes at the muscarinic receptor[1]

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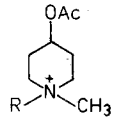
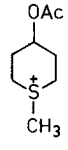
Summary. The dissociation constants (K_A) and the relative intrinsic efficacies (e) of 4-acetoxy-piperidines and -thiacyclohexanes were determined on the guinea-pig isolated left atrium. The differences in the muscarinic potencies are associated with differences in affinities and efficacies, respectively.

In previous papers from our laboratory, it has been shown that compounds **I–IV** are muscarinic agonists[3–6]. Stereochemical and thermodynamic parameters seem to be responsible for great differences in the muscarinic activity of **I–IV**.





R **I** H
II CH_3

III = CIS
IV = TRANS

As the compounds **I–IV** are agonists, 2 parameters are necessary to characterize their pharmacological action: efficacy (ability to activate the muscarinic receptor) as well as affinity. Using an ED_{50} value as an approximation of a K_A value for an agonist, a potential error can arise when there is a large receptor reserve for the agonist with respect to the response being measured. If the assumption of a receptor reserve is correct, then it is impossible to obtain truly reliable values for the dissociation constant K_A directly from agonist dose-response curves. To obtain these 'true' K_A values together with values for efficacies for compounds **I–IV**, a method has been used in this work originated by Stephenson[7] and Furchgott[8,9]. The validity of this approach has been questioned by Triggle and Triggle[10], but recent radioligand-binding studies lead to the suggestion that the K_A-values determined with such a pharmacological procedure are directly coupled to the response being measured and are true dissociation constants of the agonist-receptor complexes[11].

Methods. The experiments were performed on isolated left atria of guinea-pigs electrically driven with a frequency of 2 Hz and 3 msec duration by means of rectangular impulses of 4–6 V. Only atria from reserpinized (5 mg/kg) guinea-pigs were used[9]. The acetylcholinesterase was blocked by DFP (5×10^{-5} M), since all the agonists are substrates for this enzyme[12,15]. To block nicotinic receptors, hexamethonium (7×10^{-5} M) was used.

A fraction of the total concentration of the muscarinic receptors in the atria were irreversibly inactivated by pretreatment with dibenamine (1×10^{-4} M, 45 min). According to occupation theory, the following equation applies[8,9]:

$$\frac{1}{[A]}=\frac{1-q}{q\times K_A}+\frac{1}{q\times[A']}$$

q is the fraction of muscarinic receptors still active, [A] and [A'] are the respective concentrations of the agonists giving equal negative inotropic responses before and after inactivation with dibenamine, and K_A is the dissociation constant

Muscarinic activities of acetylcholine (Ach) and **I–IV** on the guinea-pig isolated left atrium (mean ± SE), negative effects on the force of contraction

	n*	$ED_{50}\times10^{-8}$ M**	Relative activities	$K_A\times10^{-6}$ M	Relative affinities	Relative intrinsic efficacies***	K_A/ED_{50}
Ach	12	3.08 ± 0.38	1.00	3.86 ± 0.63	1.00	1.00	125
I	8	563 ± 60	0.0055	329 ± 131	0.012	0.47 ± 0.095	58
II	8	690 ± 50	0.0045	256 ± 96	0.015	0.35 ± 0.10	37
III	8	1.60 ± 0.10	1.93	3.17 ± 0.40	1.22	1.50 ± 0.25	198
IV	8	146 ± 23	0.021	339 ± 60	0.012	1.76 ± 0.27	232

* Number of observations. ** According to Lambrecht[6]. *** The relative intrinsic efficacies were calculated from paired values between Ach and **I–IV**.

of the agonist-receptor complex. A plot of 1/[A] against 1/[A'] gives a straight line with a slope of 1/q, and the K_A-values are equal to (slope-1) divided by the intercept of the line on the 1/[A]axis. Experimental values for 1/[A] and 1/[A'] were fitted by a least-squares technique to the equation ($r = 0.974 - 0.998$). The relative intrinsic efficacies of compounds **I–IV** in relation to that of acetylcholine were determined according to the method of Furchgott and Bursztyn[8]: relative intrinsic efficacy $= e_x/e_{Ach}$.

Results and discussion. The following immediate observations can be made upon examination of the data in the table. The differences in muscarinic activity between acetylcholine and compounds **I–IV** are due to differences in the affinities to the receptor as well as differences in the efficacies. Both the sulfonium analogues **III** and **IV** possess a higher intrinsic efficacy than acetylcholine. This is the reason why their relative affinities are smaller than the corresponding relative activity values. In the case of the 2 ammonium anlogues **I** and **II**, values for relative affinities are higher than the corresponding relative activities, since these 2 drugs show lower values for their relative intrinsic efficacies than acetylcholine. However, the data in the table show some parallelism between relative activities and relative affinities, since the differences in relative intrinsic efficacies of acetylcholine and **I–IV** are not too large.

The ratio K_A/ED_{50} varies for acetylcholine and **I–IV** from 232 to 37. Data obtained by various workers[13] have shown that, with increasing potency of muscarinic agonists, the ED_{50} becomes progressively smaller than the calculated K_A-value. With the exception of the sulfonium compound **IV**, this is true for the other derivatives under investigation. Compound **III** with the highest potency possesses also the highest K_A/ED_{50} ratio. The less active ammonium derivatives show lower values for K_A/ED_{50}.

Until recently the knowledge of the mechanism of how muscarinic agents act on their receptors has been derived from an analysis of the actions of agonists on certain pharmacological responses, such as the contraction of smooth muscle and the decrease in contractile amplitude of heart muscle, respectively[11]. Quantitative parameters describing the results of such experiments are the ED_{50}, K_A-values and the efficacies, shown in the table. With the development of tritium-labelled muscarinic ligands of high specificity and high specific activity, one can now measure directly the binding of muscarinic agents to their receptors. The point of interest to emerge from these binding studies is that the binding curves respresent binding to 2 independent sites for which agonists have different affinities[11,14]. There exist 2 classes of agonist binding sites, one having a high affinity constant (K_H) and the other a low affinity constant (K_L) for agonist binding. Comparing the parameters derived from binding studies with those derived from analysis of pharmacological experiments, certain relationships emerge. There is a positive correlation between K_H and ED_{50}[14]; moreover, the absolute values tend to be similar. Values of K_L are close to the dissociation constants K_A. The question arising is, which binding site is coupled to the pharmacological response? At present, it seems to be the low affinity site K_L[11]. If this is true, the action of muscarinic agonists can be described with 2 parameters obtained in pharmacological experiments: intrinsic efficacy e – there is no capability for determining e in a radioligand procedure – and the dissociation constant K_A.

1 Structure and conformation activity relationships of heterocyclic acetylcholine analogues, VIII.

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Protein synthesis inhibition induced by dimethylnitrosamine and diethylnitrosamine on isolated rat hepatocytes

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Summary. Time- and dose-dependent protein synthesis inhibition takes place following exposure to high doses of dimethylnitrosamine (DMN) or diethylnitrosamine (DENA) in isolated rat hepatocytes. The ability of DENA to depress protein synthesis is 5-fold higher than that of DMN. Cells inhibited by 60 min exposure to DMN or DENA, and then incubated in a nitrosamine-free medium, regain their initial rate of protein synthesis. This recovery is faster and more complete for DENA-treated cells.

The i.p. injection of a single high dose of DMN or DENA in rodents produces an early and severe depression of protein biosynthesis in the liver[1,2]. Knowledge of the mechanism underlying this effect is inadequate to determine whether the nitrosamines act by inactivating some essential component(s) of the protein synthesis machinery or by interference with regulatory functions[3,4].

As a tool for studying the forward protein synthesis inhibition produced by DMN and DENA, we have used isolated rat hepatocytes prepared by an enzymatic perfusion technique[5,6], overcoming in this way many of the difficulties arising in the in vivo experiments. The viability of isolated rat liver cells is not impaired within 2 h of incubation by high doses of DMN or DENA. A time- and dose-dependent protein synthesis inhibition takes place in isolated hepatocytes treated with DMN or DENA: this effect is