of the amount synthesized in the synaptoplasm compared to only 1.5 percent present as a result of binding or contamination. That is, the amount of [^{14}C]ACh in synaptic vesicles of synaptosomes incubated with choline is greater than can be accounted for by binding or contamination from the synaptoplasmic ACh. The results suggest that ACh is synthesized in both the synaptoplasm and the synaptic vesicle. In addition, both bound and soluble choline acetyltransferase must also exist within the intact synaptosome.

Since ACh appears to be synthesized in two different pools within the nerve ending, mechanisms must exist which regulate the size of both pools. Vesicular synthesis can be regulated by product inhibition (*14*), and may account for the slower vesicular incorporation observed in our experiments. Synthesis may be further limited in vivo by the availability of substrate. Associated with neuronal activity are changes in the ionic environment of the neuron which could alter the kinetic properties of the synthesizing enzyme. Increases in the sodium concentration enhances choline acetyltransferase activity (*15*), and changes in ionic strength affect the binding of the enzyme to isolated synaptic vesicles (*5*).

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Cyclic Adenosine Monophosphate: Potassium-Dependent Action on Vascular Smooth Muscle Membrane Potential

Abstract. *Dibutyryl cyclic adenosine monophosphate and theophylline hyperpolarize smooth muscle of rabbit main pulmonary artery in low concentrations of potassium (1 millimole per liter) but do not have a significant effect on the membrane potential in the presence of high concentrations of potassium (10 millimoles per liter). The dependence of the hyperpolarizing effect on a low external concentration of potassium is similar to that observed with isoproterenol. Prior treatment with theophylline potentiated the hyperpolarizing action of isoproterenol. These findings are compatible with the assumption that potassium-dependent, beta-adrenergic hyperpolarization is mediated by cyclic adenosine monophosphate.*

Cyclic adenosine 3′,5′-monophosphate (cyclic AMP) is considered to be the secondary messenger of beta-adrenergic action in several systems (*1*). Cyclic AMP mimics the action of catecholamines in reducing the discharge frequency of Purkinje cells in the rat cerebellum (*2*). We now report the effects of cyclic AMP on the membrane potential of vascular smooth muscle. Isoproterenol, a beta-adrenergic agent, hyperpolarizes the rabbit main pulmonary artery, rat myometrium, and avian slow striated muscles when the preparations are bathed in a solution having a low external concentration of potassium (*3*, *4*). In the presence of higher external concentrations of potassium, isoproterenol does not hyperpolarize vascular smooth muscle (*4*, *5*) or avian slow muscle (*4*).

It is therefore to be anticipated that if cyclic AMP mediates the beta-adrenergic effect on the membrane potential, then the action of this nucleotide should also vary as a function of the potassium concentration of the medium and the hyperpolarizing action of isoproterenol should be potentiated by the inhibitor of phosphodiesterase, theophylline (*1*). We were able to verify both of these expectations.

Membrane potentials of strips of rabbit main pulmonary artery were determined with intracellular microelectrodes (*6*). The membrane potentials are the mean and standard error of approximately 15 penetrations for each series in each strip. The potassium concentration of the medium was either 1 or 10 mmole/liter; appropriate adjustments of sodium concentration were made to maintain isosmolarity.

Isoproterenol in a relatively low concentration of 0.12 μg/ml did not hyperpolarize the strips of artery: the membrane potentials (in 1 m*M* K^+ in Krebs solution) were 61.6 ± 1.88 mv before and 60.8 ± 2.05 mv after application of the drug (measurements in eight strips; the difference is not significant). Paired strips obtained from the same animals were first treated with theophylline for half an hour and then exposed under identical conditions to the same (0.12 μg/ml) concentration of isoproterenol. The latter now produced a very significant ($P < .001$) hyperpolarization from 59.6 ± 1.30 mv to 67.6 ± 1.65 mv ($n = 8$). In seven out of eight experiments, theophylline by

Table 1. Potassium-dependence of the action of theophylline and cyclic AMP on the membrane potential of the rabbit main pulmonary artery. Each number represents the mean membrane potential (millivolts) and standard error of approximately 15 penetrations. The bottom row shows the grand means. NS = not significant.

1 m*M* K^+			10 m*M* K^+		
Control	*P*	4 m*M* Theophylline + 1 m*M* dibutyryl cyclic AMP	Control	*P*	4 m*M* Theophylline + 1 m*M* dibutyryl cyclic AMP
59.4 ± 1.21*	$< .01$	70.2 ± 1.95	54.6 ± 1.17	NS	53.0 ± 1.36
60.1 ± 1.03*	$< .001$	70.9 ± 1.68	49.7 ± 0.46	NS	50.4 ± 0.90
62.4 ± 1.32*	$< .01$	65.5 ± 1.28	48.3 ± 0.76	$< .02$	52.4 ± 1.41
64.2 ± 1.32	$< .001$	81.3 ± 1.85	51.5 ± 1.15	NS	54.1 ± 1.62
70.4 ± 1.53	$< .001$	80.9 ± 1.82	50.8 ± 0.86	NS	53.9 ± 1.96
67.8 ± 1.46	$< .001$	77.2 ± 1.91	54.3 ± 2.02	NS	55.5 ± 0.76
64.1 ± 1.77	$< .001$	74.3 ± 2.62	51.5 ± 1.02	NS	53.2 ± 0.71

* Theophylline added 30 minutes before cyclic AMP; in all other experiments cyclic AMP and theophylline were added simultaneously and membrane potentials were measured 20 to 40 minutes after the addition of cyclic AMP.

itself had no significant effect on the membrane potential, but in one strip it produced hyperpolarization of 4 mv that was significant ($P < .05$).

Dibutyryl cyclic 3′,5′-AMP (0.5 to 1.0 mmole/liter) by itself had a significant ($P < .01$) hyperpolarizing effect in only two of seven experiments conducted in the presence of a low concentration of potassium (1 mmole/liter). In three of the above experiments, although the effect of dibutyryl cyclic 3′,5′-AMP on the membrane potential was not significant, the subsequent addition of 4 m*M* theophylline hyperpolarized these preparations. In 1m*M* K^+ solution, consistent hyperpolarization could be produced by dibutyryl cyclic AMP when the latter was preceded by a half-hour treatment with theophylline or when the nucleotide and theophylline were administered simultaneously and membrane potentials were determined within 20 to 40 minutes after addition of the drugs (Table 1). In the presence of elevated concentrations of K^+ (10 mmole/liter) the same combination of cyclic nucleotide and phosphodiesterase inhibitor did not produce a significant effect on the membrane potential (Table 1).

The potentiation of the hyperpolarizing action of isoproterenol by the inhibitor of phosphodiesterase, theophylline, is compatible with a mechanism mediated by cyclic 3′,5′-AMP. The similar dependence on potassium of the electrogenic actions of the cyclic nucleotide and of isoproterenol further supports this mechanism of action. In similar experiments, adenosine monophosphate (in 1 m*M* K^+ solution) did not hyperpolarize the rabbit main pulmonary artery, indicating that the effect of the cyclic nucleotide is specific. The relatively high concentrations of cyclic nucleotide required for hyperpolarization and the potentiation of this effect by theophylline suggest that the site of hyperpolarizing action is the inner, or at least not a readily accessible, surface of the cell membrane.

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Chromosomal Aberrations Induced in Barley by LSD

Abstract. *Seeds of hulled barley* (Hordeum vulgare) *were germinated and then treated with LSD. Preparations of squashed root tips stained with Feulgen revealed extensive chromosomal aberrations, most of which were chromosome breaks. Nearly half of the breaks occurred in the region of the primary constriction.*

There has been considerable interest in the possible genetic effects of LSD (lysergic acid diethylamide), and a number of conflicting reports have appeared. Most of these reports have been summarized recently by Sturelid and Kihlman (*1*), who themselves studied the effects of various doses of this drug on broad bean cells, Chinese hamster cells, and human leukocytes. Like several of the earlier authors (*2*), Sturelid and Kihlman (*1*) could find no evidence that LSD induces chromosome aberrations in their material. On the other hand, Cohen *et al.* and Skakkebaek *et al.* observed different types of chromosomal aberrations in human peripheral leukocytes (*3*) and in mice (*4*) treated with LSD.

We have studied the effect of LSD on chromosomal structure of barley. Seeds of hulled barley variety NP 113 (*Hordeum vulgare*, $2n = 14$) were germinated overnight at 25°C and then treated with an aqueous solution of LSD (Sandoz, 25 μg/ml) for either 4 or 8 hours. The treatments were followed by 4- or 8-hour recovery periods, after which the root tips were treated with 0.1 percent colchicine for 1 hour and then fixed in a mixture of ethanol and acetic acid (3 : 1). The preparations of squashed root tips were stained with Feulgen by the normal procedure. Chromosomal aberrations were scored in well-spread metaphase cells as described by Darlington and La Cour (*5*).

There was evidence of extensive

Table 1. Observations on aberrations induced with LSD, including chromosomal (B″) and chromatid (B′) breaks. The mean values are based on the scoring of total number of cells including those not showing aberrations.

Treatments			Metaphase cells scored (No.)	Aberrant cells (%)	Mean number of different chromosomal aberrations per cell		
LSD (μg/ml)	Time (hr)	Recovery (hr)			B″	B′	Achromatic gaps
0	4	0	180	0.00	0.00	0.00	0.00
25	4	4	250	37.69	2.20	.50	
25	4	8	201	44.80	3.00	.66	.74
0	8	0	290	1.63	0.08	.04	
25	8	4	131	46.34	3.05	.61	.04
25	8	8	209	56.16	4.03	.77	.10

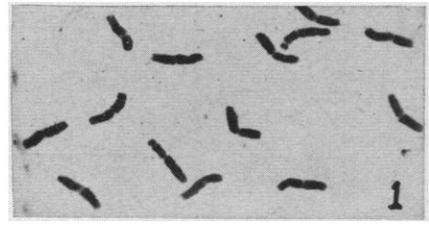

Fig. 1. Metaphase cell showing the normal complement of 14 chromosomes in the control material (× 1800). Fig. 2. Metaphase cell from root tip treated with LSD, showing extensive chromosomal damage (× 2500).