Zur Prüfung dieser Annahme wurden Versuche an Nerv-Muskelpräparaten des Soleus und Tibialis anterior der Katze *in situ* durchgeführt. Die Muskeln wurden indirekt mit supramaximaler Reizstärke und Rechteckimpulsen verschiedener Frequenzen gereizt. Die Registrierung der Muskelspannung erfolgte isometrisch mit einem Dehnungsmeßstreifen.

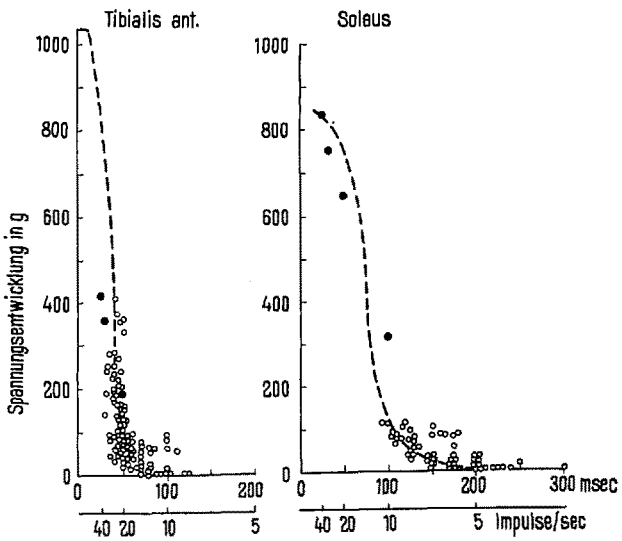

Fig. 2. Kurven des aktiven Zustandes des Tibialis anterior und Soleus. Die mit der Methode der schnellen Entlastung gewonnenen Werte sind durch Kreise wiedergegeben; sie wurden der Arbeit von JURNA et al.[11] entnommen. In diese Diagramme wurden als dicke Punkte die Mittelwerte der Spannungsentwicklung aus der Figur 1 gegen das Reizintervall (Kehrwert der Reizfrequenz) aufgetragen. Ordinate: Spannungsentwicklung in g. Abszissen: Zeit in msec als Zeit nach dem Einzelreiz (für die Methode der schnellen Entlastung) oder als Reizintervall (bei repetitiver Reizung) und Reizfrequenz in Impulsen/sec.

Die Kurven der Spannungen während maximaler Summation sind in Abhängigkeit von der Reizfrequenz wiedergegeben (Spannungs-Frequenzdiagramme, Figur 1). Trägt man die ermittelten Spannungen nicht gegen die Frequenz, sondern gegen das entsprechende Impulsintervall auf, so ergibt sich eine gute Übereinstimmung mit den Werten, die mit der Methode der schnellen Entlastung gewonnen wurden[11]. Darüber hinaus kann man mit Hilfe der so gewonnenen Punkte den Verlauf der Erschlaffungskurve der kontraktilen Elemente auch im ersten Teil nach dem Plateau festlegen (Figur 2). Dies ist hier nur beim Soleus durchgeführt worden, prinzipiell aber auch beim Tibialis anterior möglich. Das Ende des aktiven Zustandes lässt sich, wie früher gezeigt[11], dadurch bestimmen, dass man das Reizintervall ermittelt, bei dem noch keine Summation auftritt. Die auf diesem Wege erhaltenen Werte stimmen mit denen überein, die durch Anwendung der Methode der schnellen Entlastung gewonnen wurden.

Summary. By plotting the tension developed during maximal summation of muscle twitches against the stimulus interval, a curve can be drawn which has the same time course of the falling phase of the active state as determined by the method of quick release.

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Regional Changes of Acetylcholine and Choline Acetylase Activity in the Guinea-Pig's Brain after Scopolamine

ITOH et al.[1] found that Dopa-decarboxylase, Dopamine-β-oxidase and monoamine-oxidase activity of the guinea-pig's brain significantly increases 2-4 h after reserpine treatment. This observation suggests that the enzymatic induction may be very fast in the brain.

Up to the present time, little attention has been paid to the changes of choline acetylase activity after drugs able to modify the acetylcholine levels. We shall now describe the changes of acetylcholine content and of choline acetylase activity in different areas of the guinea-pig's brain, after acute and subacute treatment with scopolamine, which is known to reduce cerebral acetylcholine[2].

1, 5, and 25 mg/kg of scopolamine were given i.p. to guinea-pigs of both sexes weighing 250–350 g. The animals were decapitated at a given time and their olfactory lobes, superior parietal cortex, caudate nucleus, thalamus, and cerebellar vermis quickly removed. Total acetylcholine was extracted with our method[3], and biologically estimated on eserinized frog rectus muscle against suitable standards. Choline acetylase activity was determined at 37°C, according to the method of BULL et al.[4]; total cholinesterase activity was also determined at 37°C, according to AMMON's method[5].

Moreover, scopolamine cerebral concentrations were estimated by assaying the antagonism of the brain extracts and of the drug standard solutions against acetylcholine test doses, on the guinea-pig's terminal ileum.

As shown in Table I, total acetylcholine in olfactory lobes, parietal cortex and caudate nucleus is significantly reduced as early as 20 min after 25 mg/kg of scopolamine; it approaches the control values after 3 h. No change takes place in the thalamus and cerebellar vermis. Similar effects are observed after 5 mg/kg of scopolamine (values not given), but there is no statistical difference between controls and 1 mg/kg treated animals.

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Table I. Acetylcholine content ($\mu g/g \pm$ S.D.) in different areas of guinea-pig's brain, after scopolamine 25 mg/kg, i.p. (acute and subacute treatment). In brackets, the number of experiments

Area	Controls	Acute treatment			Subacute treatment	
		After 20 min	After 1 h	After 3 h	24 h after the 7th dose	1 h after the 8th dose
	(40)	(14)	(14)	(12)	(10)	(11)
Cerebral cortex	2.670 ± 0.58	1.530 ± 0.54[a]	1.810 ± 0.28[a]	2.140 ± 0.48[b]	3.120 ± 0.52[b]	1.630 ± 0.39[a]
Olfactory lobes	2.090 ± 0.55	1.760 ± 0.46[b]	1.880 ± 0.40	2.280 ± 0.27	2.400 ± 0.45	1.910 ± 0.39[c]
Caudate nucleus	4.820 ± 0.97	3.590 ± 0.74[a]	3.730 ± 0.57[a]	4.020 ± 0.62[b]	6.580 ± 1.37[a]	3.850 ± 0.69[b]
Thalamus	5.320 ± 0.97	5.360 ± 0.74	5.640 ± 0.51	5.030 ± 0.82	5.150 ± 0.87	5.030 ± 0.82
Cerebellar vermis	0.539 ± 0.142	0.542 ± 0.087	0.612 ± 0.062	0.612 ± 0.126	0.613 ± 0.100	0.632 ± 0.125

[a] These values differ significantly from the controls at a level of $P < 0.001$. [b] These values differ significantly from the controls at a level of $P < 0.01$. [c] This value differs significantly from the seven-day treated group at a level of $P < 0.01$.

Table II. Choline acetylase activity (formed ACh $\mu g/g$ of fresh tissue h, $\pm$ S.D.) in different areas of guinea-pig's brain, after scopolamine 25 mg/kg, i.p. (acute and subacute treatment). In brackets, the number of experiments

Area	Controls (13)	Acute treatment		Subacute treatment
		After 1 h (7)	After 3 h (7)	24 h after the 7th dose (8)
Cerebral cortex	893 ± 104	895 ± 264	1293 ± 279[a]	1325 ± 223[a]
Olfactory lobes	675 ± 114	685 ± 124	690 ± 108	893 ± 155[a]
Caudate nucleus	5093 ± 934	5414 ± 735	5171 ± 790	6750 ± 936[a]
Thalamus	1206 ± 201	1139 ± 282	1153 ± 300	1253 ± 237
Cerebellar vermis	92 ± 17	92 ± 27	80 ± 22	97 ± 15

[a] These values differ significantly from the controls at a level of $P < 0.001$.

In seven-day treated guinea-pigs, the total acetylcholine in the olfactory lobes, parietal cortex and caudate nucleus is above the normal values 24 h after the seventh injection. However, the depleting effect of the drug is still present 1 h after the eighth dose. No significant change is detected in the thalamus and cerebellar vermis even after subacute treatment.

As shown in Table II, choline acetylase activity is unmodified 1 h after the first injection of the drug; it increases significantly 3 h later in the parietal cortex only. However, after a seven-day treatment, the enzymatic activity in the olfactory lobes, parietal cortex and caudate nucleus is higher than in normal animals. At this moment, as described above, the amount of total acetylcholine in the same areas is greater than in the controls. No further increase of enzymatic activity is detectable in the parietal cortex 3 h after the eighth dose.

Total cholinesterase activity is never modified in any area during acute or subacute treatment. The normal values, expressed in mg of split acetylcholine/g fresh tissue/h, are: 26.8 ± 3.11 in the olfactory lobes, 34.4 ± 3.04 in the parietal cortex, 172.5 ± 18.3 in the caudate nucleus, 65.1 ± 7.5 in the thalamus, and 122.9 ± 12.7 in the cerebellar vermis (average of 20 experiments ± S.D.).

The scopolamine concentration of the whole brain is very high 20 min after 25 mg/kg (1090 ± 79 ng/g, 8 experiments); it falls to 378 ± 63 ng/g after 1 h, and has the same value at the third hour, 362 ± 82 ng/g.

Therefore, the scopolamine tissue concentration and the acetylcholine depletion seem to have different time courses; this may be accounted for by the increased synthesis rate.

Our findings show that biochemical changes of the brain's cholinergic system after scopolamine are restricted to only certain cerebral areas: they concern the acetylcholine levels and the synthetic (but not esterasic) activity. The increase of choline acetylase, which follows the acetylcholine depletion, suggests that the drug enhances the acetylcholine release instead of inhibiting its synthesis. Recent histochemical investigations[6,7] show that cholinergic fibres are present inside the cerebral cortex and between different structures of the forebrain: it is likely that scopolamine stimulates, by an unknown mechanism, only this neuronal pool. Our hypothesis agrees with the electrophysiological findings, supporting the concept that scopolamine and allied drugs act at the higher levels of neuronal integration[8].

Riassunto. La scopolamina riduce la acetilcolina totale nella corteccia cerebrale, bulbo olfattorio, nucleo caudato, ma non nel talamo e cervelletto. La attività colinacetilasica aumenta nelle zone dove si manifesta la riduzione di neurormone. Ciò suggerisce che la scopolamina stimoli soprattutto strutture colinergiche telencefaliche.

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