L'accoutumance aux excitants étudiés s'effectue par la disparition des troubles respiratoires et des mouvements de l'animal dans la cage actographique, mais aussi par une augmentation marquée des groupements –SH non-protéiques (+ 35,8%) et une baisse accentuée des groupements –SH totaux et protéiques (– 12 à – 18,9%; $P < 0{,}01$). En ce qui concerne la glycémie, on assiste à une tendance continue vers la normale, mais même chez l'animal déjà accoutumé elle reste plus élevée (+ 13%) que la normale ($0{,}02 > P > 0{,}01$).

Nous ne pouvons pas encore donner une intérprétation satisfaisante des phénomènes observés. Mais l'augmentation progressive des groupements –SH non-protéiques, pendant le déroulement d'accoutumance à un excitant nouveau, nous croyons pouvoir l'attribuer à la dénaturation réversible des protéines, phénomène décrit aussi par NASONOV[12] et UNGAR[13].

En conclusion, nous avons constaté que le reflexe d'orientation à un nouvel excitant détermine une augmentation de la glycémie et des groupements –SH non-protéiques du sang, ainsi qu'une baisse des groupements –SH totaux et protéiques. Pendant la disparition du reflexe d'orientation à la suite de l'accoutumance, les groupements –SH non-protéiques restent les plus affectés.

Summary. The orientation reflex, to a new milieu, causes increase of the glycemia and the non-protein –SH groups, and a diminution of the protein and total –SH groups of rabbit's blood.

E. A. PORA, MIRCEA POP, et N. FABIAN

Chaire de Physiologie animale de l'Université de Cluj (Roumanie), le 21 décembre 1964.

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Transmitter Release in the Rat Diaphragm During Tetanic Nerve Stimulation

In curarized mammalian muscle several investigators have observed a progressive decline in repetitively evoked endplate-potentials (EPPs) from the onset of the tetanic stimulation period[1–3]. As the sensitivity of the endplate membrane to acetylcholine (ACh) seems to be unchanged during short periods of repetitive transmitter action[4,5], and as the EPPs seem to be approximately proportional to the amount of transmitter liberated[6], this amplitude reduction is usually presumed to indicate a diminution of the per impulse release of ACh from the nerve terminals[4,7,8]. This has been interpreted as a physiologically significant phenomenon[4,6], a view which, however, has been called in question by NAESS[8], since the decline in EPPs is seen in preparations pretreated with *d*-tubocurarine, a substance which seems to interfere with presynaptic events[9–12]. (For a more detailed discussion see LILLEHEIL and NAESS[9].) When high magnesium or low calcium concentrations are used to prevent the initiation of muscle contraction, there is on the contrary an initial potentiation of successive EPPs in a tetanic train. A reasonable explanation of this observation seems to be that, due to high magnesium and low calcium concentration, a smaller fraction of the available ACh is released by each impulse than in the normal or curarized preparation. The nerve terminals are thus spared for the serious transmitter depletion from which they probably suffer in curarized preparations[5,6]. This hypothesis presupposes that the amount of available ACh is roughly the same in the different experimental situations mentioned. The mechanisms concerned with mobilization and release of transmitter are most probably influenced by the substances used. An attempt was therefore made to investigate tetanic series of EPPs without adding any modifying agents. This is described cursorily in the following.

Using the method first described by BARSTAD[11], in which the muscle fibres of an isolated rat diaphragm strip were cut transversally on either side of the endplate region, it is possible within a limited time period (about 30–120 min after cutting) to make intracellular recordings of EPPs without the disturbing effect of muscle contractions (Figures 1, 2, and 3). The following criteria show that the recorded potentials really are EPPs: (i) Miniature-endplate potentials (mEPPs), indicating that the electrode is standing at or close to the endplate. (ii) Summation of two impulses in quick succession. (iii) Elongation of the potentials (Figure 3 B and C) after addition of an acetylcholinesterase-inhibitor (prostigmine).

Figure 1 shows recordings of indirectly evoked trains of EPPs at 50/sec (B) and 100/sec (C) lasting about 5 sec each. The initial phase of each train is characterized by a 10–20% fall in the EPP amplitude followed by a gradual increase, which to some extent depends on the stimulation frequency. On further stimulation there is a gradual decline in the amplitude of the EPPs, which reaches half the original value in about 5 min at a frequency of 50/sec (Figure 2).

It was checked at higher sweep velocity that no presynaptic failure[13] was present. From fibre to fibre, only

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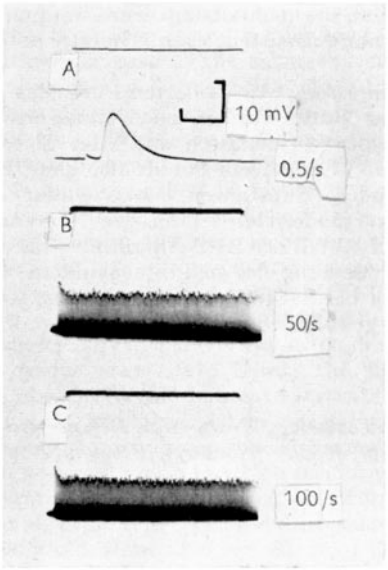

Fig. 1. Intracellular recordings of membrane and endplate-potentials (EPPs) from isolated rat diaphragm in Tyrode solution at 37°C after transversal cutting of the muscle fibres on either side of the endplate region. – The approximate length of the muscle fibres after cutting was 5 mm. A glass microelectrode filled with 3 molar KCl solution and with a resistance of 10 MΩ was used. The phrenic nerve was stimulated with supramaximal rectangular pulses of 0.05 msec duration. The uppermost trace in each recording represents the zero reference (the potential of the bath fluid). In A, membrane potential and EPP 70 min after cutting. Stimulation frequency, 0.5/sec. In B and C, trains of EPPs at frequencies of 50/sec and 100/sec respectively. B recorded 1 min after A, C 2 min after B. Voltage scale: 10 mV. Time scale: 2 msec (A) and 1 sec (B and C).

minor variations were seen in this pattern, which was unaffected by change of bath fluid from Tyrode (Figure 1) to Ringer-Locke (Figure 2) solution. The observations are in agreement with the findings of LILLEHEIL and NAESS[9] that the amplitude of the extracellular EPPs recorded in the introductory phase of curarization are well maintained during short periods of tetanization.

It is assumed that after cutting and subsequent gradual depolarization, the permeability change in the endplate-membrane caused by a certain amount of ACh is approximately normal. The reduced amplitudes of the EPPs are probably mainly due to the altered ionic distribution with reduced electrochemical driving forces. Therefore the postsynaptic mechanism of the EPP reduction in this case certainly differs fundamentally from the mechanism of the postsynaptic curare action. This difference, however, should be of minor importance as long as the amplitudes are taken as indications only of relative amounts of ACh liberated per impulse in a tetanic train.

The possibility exists that the nerve terminals could be influenced by altered conditions brought about by the cutting procedure. In some experiments a reduction in the size of the EPPs with no parallel decrease in the membrane potential has been observed during low frequency stimulation (0.5/sec). This could possibly be explained as a pre- or postsynaptic side effect of the electrode[13], but could also indicate a reduced per impulse output of ACh corresponding to a diminished nerve spike amplitude[14], which in turn might be due to a presynaptic depolarizing effect of the muscle demarcation. A presynaptic effect of the cutting is also indicated by the observation made by RANDIĆ and STRAUGHAN[15] of an increased mEPP frequency 4–40 min after the cutting. Collecting ACh over

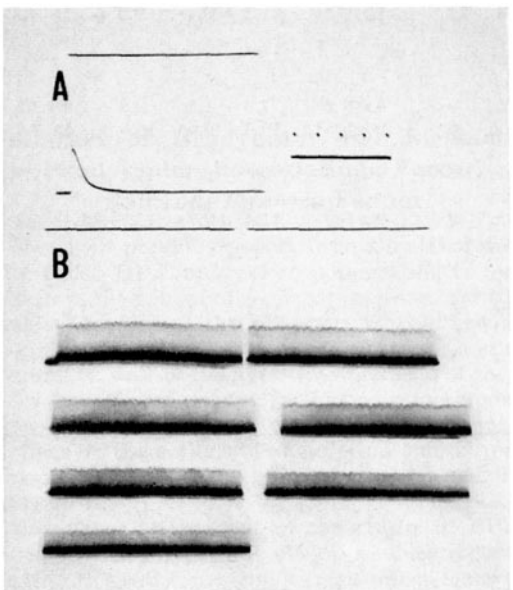

Fig. 2. Procedure as in Figure 1, except for use of Ringer-Locke solution. In A, membrane potential and EPP 50 min after cutting the muscle fibres. Stimulation frequency, 0.5/sec. In B, seven fragments, each of approximately 8 sec duration, from a continuous recording of EPPs at a frequency of 50/sec. The upper two horizontal trains with zero reference line represent the first 16 sec of the stimulation period. Then follows below from left to right (without zero reference) fragments taken 1, 2, 3, 4, and 5 min after the onset of stimulation. Time scale: 2 msec (A) and 1 sec (B). Voltage scale: 5 mV (the amplitude of the time marker pulses).

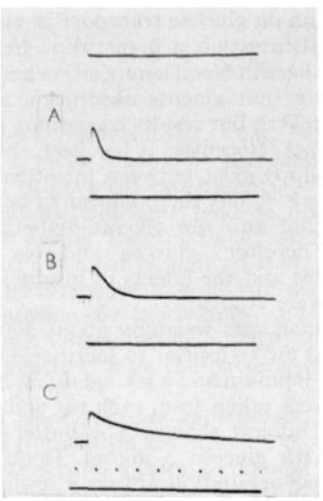

Fig. 3. Effect of prostigmine on the recorded potentials. From the same muscle fibre as in Figure 2 approximately 10 min after the end of the tetanic train. Stimulation frequency, 0.5/sec. In A, recording before prostigmine addition. In B and C, recordings 1 and 2 min respectively after the addition of prostigmine bromide, 2 μg per ml bath fluid. Note the elongation of the potential. Time scale: 2 msec. Voltage scale: 5 mV (the amplitude of the time marker pulses).

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20 min periods during stimulation at a frequency of 20/sec, the same authors found a reduced rate of ACh liberation the first $^1/_2$ h after cutting. After that time, the ACh liberation returned to approximately the precutting value. These temporary effects can probably be avoided when recordings are made at later stages after cutting (Figures 1 and 2). On the other hand, HUBBARD and WILLIS[3] demonstrated that presynaptic hyperpolarization, though increasing the absolute EPP amplitudes, did not substantially counteract the progressive decline of these amplitudes in curarized preparations. This seems to rule out hyperpolarization as an explanation of the differences between the tetanic trains here presented (Figure 1 B and C, and Figure 2 B) and those seen during later stages of curarization.

If the records of the tetanic EPP trains presented above give a correct picture of the normal course of transmitter release during tetanization, the mechanisms involved in this release at the mammalian neuro-muscular junction seem to be more persistent than generally assumed.

Zusammenfassung. Am isolierten Phrenicus-Zwerchfellpräparat der Ratte wurden, nach transversaler Durchschneidung der Muskelfasern auf jeder Seite der Endplattenregion, Endplatten-Potentiale, ohne Zusatz von modifizierenden Substanzen, intrazellulär registriert. Während kurzdauernder tetanischer Nervenreizung erwies sich der Abfall der EPP-Amplituden als unerheblich und die Freisetzung des neuro-muskulären Überträgers Acetylcholin bei Säugetieren während des Tetanus, entgegen der gewöhnlichen Auffassung, als offenbar anhaltender.

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Intestinal Transport of Glucose and Sodium: Changes in Alloxan Diabetes and Effects of Insulin

SOLS[1] has reported that insulin stimulated glucose absorption from intestinal loops, but it has been suggested that changes in blood sugar levels may have been responsible for the effect[2,3]. Insulin in vitro failed to increase galactose uptake by rings of hamster intestine[4]. In view of these conflicting reports, it seemed of interest to study effects of insulin on glucose transport in vitro by everted segments of rat intestine, a preparation free from the influences of changes in blood composition and flow. Several reports indicate that glucose absorption is increased in alloxan-diabetes[5–7], but results concerning effects of insulin again conflict. Recently, it has been shown that intimate relationships exist between intestinal transport of Na and glucose[8–10], but there appear to be no reports on effects of insulin and the alloxan-diabetic state on Na absorption. Therefore, glucose and Na transport in alloxan-diabetes and the effects of insulin in normal and diabetic rats were studied.

Male Holtzman rats weighing about 300 g were used. All were fasted for 18 h prior to sacrifice. One 15 cm segment of upper jejunum and a second of lower jejunum and upper ileum were taken from each rat and incubated for 90 min in a Dubnoff shaker. The buffer was a Krebs-bicarbonate with glucose 3 mg/ml. Details of segment preparation and incubation appear elsewhere[11]. Alloxan was given by tail vein at a dose of 40 mg/kg after a 24 h fast, blood sugar determined one week later and rats with values above 300 mg% considered diabetic. All were sacrificed 13 or 14 days after alloxan. Insulin was administered in divided doses, half as PZI 18 h before sacrifice and the rest as Lente Iletin 1 h before sacrifice. Glucose in the serosal (absorbed) fluid was determined by the SOMOGYI method[12] and Na by flame photometry. Segments were dried to constant weight and absorption in μg/mg tissue dry weight determined.

Effects of insulin in normal animals appear in the Table. The term 'absorption' means net transfer from mucosal (outer) fluid to serosal fluid. The hormone caused a marked increase in glucose absorption and a small de-

Effects of insulin and alloxan diabetes on Na and glucose transport

Group	No. of segments	Glucose absorption (μg/mg/h)	Serosal fluid glucose concentration (mg/ml)	Na absorption (μg/mg/h)	Fluid absorption (mg/mg/h)
Normal	11	48 ± 6	7.4 ± 0.6	11.8 ± 1.1	4.3 ± 0.3
Normal insulin 0.75 μ	11	80 ± 5^a	11.3 ± 0.4^a	9.0 ± 1.1^a	4.2 ± 0.4
Normal	6	45 ± 8	7.9 ± 1.5	9.8 ± 2.3	3.5 ± 0.8
Diabetic	6	96 ± 5^a	10.8 ± 0.6^a	19.0 ± 2.1^a	7.0 ± 0.7^a
Diabetic	6	96 ± 8	9.6 ± 0.6	19.1 ± 1.5	7.9 ± 0.6
Diabetic insulin 0.75 μ	8	96 ± 9	11.4 ± 0.7^a	13.7 ± 1.1^a	6.2 ± 0.5^a
Diabetic	6	80 ± 6	11.1 ± 0.3	12.5 ± 1.6	5.2 ± 0.5
Diabetic insulin 2.50 μ	8	76 ± 7	12.5 ± 0.5^a	9.5 ± 0.6^a	4.0 ± 0.3^a

Mean $\pm$ S.E. $^a P < 0.05$.

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