Übersicht der Enzymaktivitäten (in mU/ml)

Enzyme		Ruhewerte n	$\bar{x}$	s%	Höchste Belastungswerte Zeit Belastung (h)	n	$\bar{x}$	s%	t-Test
AP	♂	29	23,84	40,67	0,5	29	28,35	35,80	0,45*
CK	♂	24	901,16	41,98	4	27	1947,57	59,45	4,65***
ALD	♂	32	36,74	42,05	24	28	123,14	28,09	12,60***
LDH	♂	30	2040,41	50,54	8	34	4949,26	57,82	5,26***
LDH1	♂	27	399,51	58,08	24	32	1718,26	55,96	6,80***
AP	♀	29	27,74	36,79	0,5	22	39,05	39,10	3,17**
CK	♀	24	676,28	51,84	1	31	1370,85	57,02	4,09***
ALD	♀	32	26,25	53,94	24	34	61,20	49,67	5,92***
LDH	♀	25	984,28	59,34	8	35	3365,42	47,19	7,14***
LDH1	♀	26	190,06	54,77	8	32	780,83	52,24	7,22***

* n.s., $p > 0,05$; ** $p \leq 0,01$; *** $p \leq 0,001$.

ausgeblutet. Die weiteren Gruppen wurden nach 0,5, 1, 2, 4, 8 und 24 h der Belastung ausgeblutet. Das Blut wurde in heparinisierten Reagenzgläsern aufgefangen, zentrifugiert und das Plasma bei −30 °C aufbewahrt.

Mit den Testkombinationen der Firma Boehringer, Mannheim, wurden die Aktivitäten in mU/ml der folgenden fünf Enzyme ermittelt: 1. Alkalische Phosphotase (AP), 2. Creatin-Kinase (CK), 3. Fructose-1,6-diphosphat-Aldolase (ALD), 4. Lactat-Dehydrogenase (LDH), 5. Lactat-Dehydrogenase-1 Isoenzym = α-Hydroxybutyrat-Dehydrogenase (LDH 1).

In den Abbildungen 1–5 sind die Ergebnisse, getrennt nach den Geschlechtern, grafisch aufgezeichnet, da in der gleichen Population signifikante geschlechtsspezifische Enzymaktivitätsunterschiede gefunden wurden[7]. In der Tabelle sind die Ruhewerte und die maximalen Ausschüttungswerte, sowie die statistische Sicherung durch t-Test zwischen den beiden Werten, wiedergegeben. Zwischen den Ruhewerten und dem Maximum der Enzymaktivitäten, bis auf AP bei den männlichen Tieren, sind signifikante Unterschiede festzustellen. Bei AP beider Geschlechter wurde 0,5 h nach der Belastung die höchste Aktivität registriert. 4 h nach der Belastung sank die Aktivität bereits unter den Ruhewert und erreichte nach 8 h den signifikant niedrigsten Wert von $\bar{x}_{♂} = 19,18$ mU/ml bei ($t = 2,41$*) und $\bar{x}_{♀} = 22,23$ mU/ml bei ($t = 2,80$**). Nach 24 h ist ein leichter Anstieg der Aktivitäten zu beobachten, die Werte blieben jedoch weiterhin unter dem Ruhewert.

Der maximale CK-Wert wurde bei den männlichen Tieren 4 h nach der Belastung erreicht, bei den weiblichen bereits nach 1 h. Im weiteren Zeitverlauf ist ein leichter Abfall der Aktivitäten festzustellen. 24 h nach der Belastung lagen die Werte $\bar{x}_{♂} = 1160,83$ mU/ml ($t = 1,63$ n.s.) und $\bar{x}_{♂} = 1007,20$ mU/ml ($t = 2,93$**) noch über den Ruhewerten.

Aldolase zeigt nach der Belastung einen permanenten Anstieg in der Aktivität. 24 h nach der Belastung wurden die höchsten Aktivitäten gemessen.

Die Aktivität des Enzyms LDH hat ihr Maximum 8 h nach der Belastung erreicht. Im weiteren Zeitverlauf zeichnet sich ein leichter Abfall der Aktivitäten ab, die jedoch 24 h nach der Belastung noch immer signifikant über den Ruhewerten bleiben.

Bei LDH 1 hatten die männlichen Tiere erst nach 24 h den maximalen Wert erreicht, während die weiblichen Tiere bereits 8 h nach der Belastung, entsprechend dem Enzym LDH, den höchsten Wert zeigten. Ungeachtet der unmittelbaren physiologischen Ursachen des Enzymanstieges im Blutplasma, kann bei den 5 untersuchten Enzymen festgestellt werden, dass innerhalb der ersten 24 h nach einer definierten Belastung im Vergleich zu Ruhewerten ein signifikanter Anstieg der Aktivität stattgefunden hat. Ihr Wert als Indikator einer stattgefundenen Belastung ist davon abhängig, in welcher Zeit nach der Belastung ihre Aktivität im Blutplasma kontrolliert wird.

1 P.D. Griffiths, Clin. chim. Acta *13*, 413 (1966).
2 D.A. Cunningham and J.B. Critz, Int. Z. ang. Physiol. *30*, 302 (1972).
3 T.K. Mukherjee, F. Major, E.S. Tawfik und P. Horst, Z. Tierzücht., Zücht. Biol. *93*, 48 (1976).
4 K. Bickhardt, Proc. 2nd Symp. Condition Meat Quality Pigs, 36 (1971) Res. Inst. f. Anim. Husb. «Schoonoord» Zeist.
5 L. Richter und A. Willeke, D. Gefl. wirt. Schw. Prod. *37*, 922 (1974).
6 J.-H. Weniger, P. Horst, D. Steinhauf, F. Major, M. Wolf und E.S. Tawfik, Z. Tierzücht., Zücht. Biologie *91*, 265 (1974).
7 F. Major und E.S. Tawfik, Z. Tierzücht., Zücht. Biol., in Druck (1978).

The influence of GABA on discharges of cortical epileptogenic focus

P. Mareš, J. Mareš, P. Remeš, M. Hessler and J. Fischer

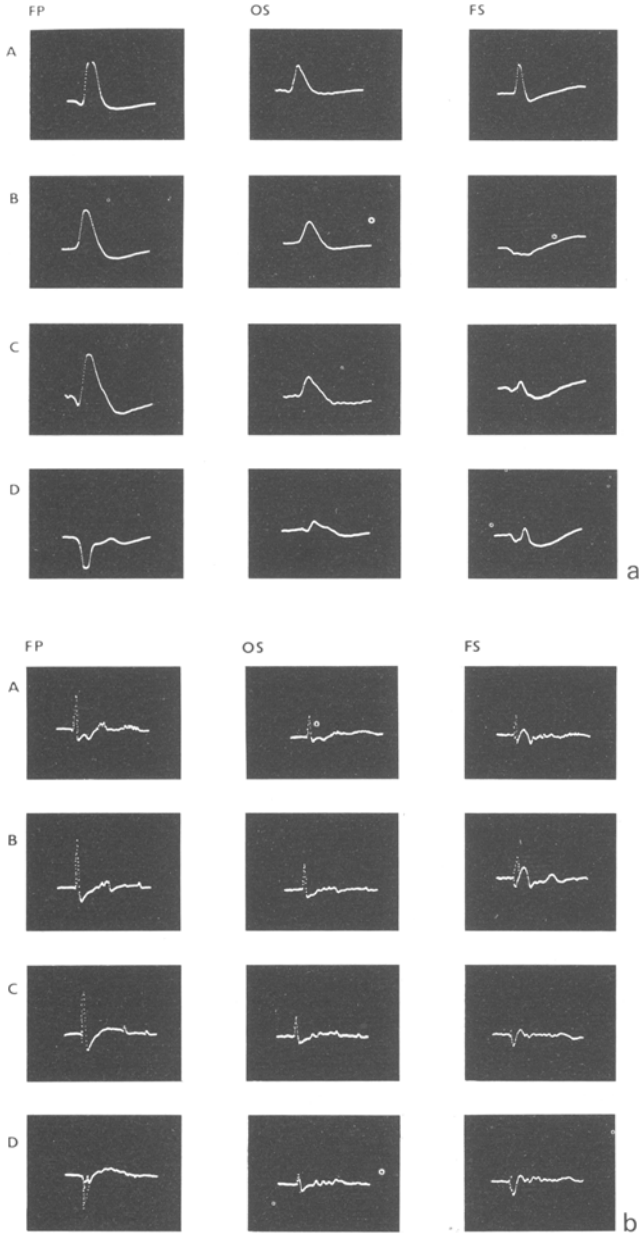
Institute of Physiology, Czechoslovak Academy of Sciences, Budějovická 1083, CS-142 20 Prague 4, and Institute of Physiology, Faculty of General Medicine, Charles University, Prague (Czechoslovakia), 4 April 1978

Summary. GABA, when applied locally, acted similarly on both primary and mirror cortical focus: the negative component of the spike discharge was suppressed or inverted in polarity, whereas the late slow negative wave was strongly potentiated. Recordings from deep cortical layers suggested a different origin of these 2 surface-negative components of focal discharges.

Gamma-aminobutyric acid (GABA), one of the inhibitory transmitters in the central nervous system[1], has a marked effect on the activity of individual neurones as well as on evoked potentials[2]. The role of GABA in epilepsy has recently found much attention[3,4]. We studied the influence of GABA upon cortical epileptogenic focal discharges, in order to determine a possible direct effect upon primary focal activity, as well as for studying individual components

in primary and projected discharges. Part of the results appeared earlier in abstract form[5].

21 adult male rats of the Wistar strain were prepared under ether anaesthesia (trephine openings and tracheal cannula); then wounds and pressure points throughout were infiltrated with 1% procaine. The ether anaesthesia was then interrupted and the rats immobilized with d-tubocurarine. Epileptogenic foci were produced by applying small amounts of sodium salt of penicillin (PNC) upon the undamaged dura mater on the sensorimotor (15 rats) or the visual (6 rats) cortical areas. The findings obtained for both cortical areas did not appear to differ.



a Averaged discharges of the PNC focus in the frontal, sensorimotor cortical region. FP, primary focal activity; OS, secondary projected focal activity in the occipital region of the same hemisphere; FS, activity of a mirror (secondary) focus in the frontal region of the opposite hemisphere. A, control; B, after GABA application to mirror focus; C, control after rinsing off with physiological solution; D, after GABA application to primary focus. Activity is in reference connection, upward deflection indicates negativity of cortical electrode. The length of the curve is 256 msec. *b* Same conditions, but length of curve is 2048 msec.

The electrocorticogram was recorded from the immediate vicinity of the application site (primary focus), from another cortical region in the same hemisphere and from the opposite hemisphere from the point symmetrical to the primary focus (projected, mirror focus). After the stabilization of focal activity, usually 10 min after PNC application, we applied a filtration paper (1.5 × 1.5 mm) saturated with a 1.5% solution of GABA to the primary of mirror focus. The recording procedure was described previously[6].

Primary focal discharges were formed by a triphasic positive-negative-positive spike in which the negative component dominated in size; rhythmic after-discharges were frequently observed. In comparison with the primary discharges, mirror focal discharges were of shorter duration and their amplitude was lower; after the spike, there always followed a late negative wave, and only after it the rhythmic after-discharge which was more marked than in the primary focus (figures, A).

After application of GABA upon the primary focus (in 8 rats at 1st application, in 13 rats after the application of GABA and rinsing the mirror focus with saline solution), the frequency of focal discharges always tended to decrease, but the difference did not reach statistical significance. However, the waveform of the discharge changed. The triphasic spike was transformed into a broad positivity, a negative component might remain merely as a notch on the ascending part of the surface positivity which was followed by a slow negative wave. Mirror discharges did not reveal obvious alterations, even if the primary focal discharges were profoundly altered (figures, C, D).

During the application of GABA on the mirror focus (13 rats), the dominant negative phase of the spike was suppressed, or more often its polarity inverted (figures, B). Marked increase in amplitude and duration of the late negative wave was always a first sign of a GABA effect. Rhythmic after-discharges as late components of focal discharges were present even after the application of GABA.

Thus GABA appears to suppress the negative spike component of both primary and projected discharge and provoked a large late negative wave, regardless of whether such a wave was present before application (in mirror foci) or not (in primary foci). In another 7 rats with PNC foci in sensorimotor region, we therefore registered in the mirror focus not only surface activity, but also activity in deeper layers of the cerebral cortex. For this we used 3 metal semimicroelectrodes (tip diameter approximately 30 µm) glued together in such a way that individual tips were vertically spaced at 400 µm. Small electrolytic lesions, marking the position of the deepest electrode, were found in 4 out of 7 rats at the identical depth of 1000–1100 µm, i.e. in the 5th layer, while the other 2 electrodes were located in depths of about 600–700 and 200–300 µm. In reference connection, a positive deflection corresponded to the surface negative phase of the spike in cortical depth of 1000 µm. Under the influence of GABA, the polarity changed only in the superficial layers while the deepest electrode did not record differences in the shape of the spike before and after GABA application. The late negative wave appeared with unchanged polarity at all depths studied, and was augmented after the application of GABA both in the superficial as well as deep layers of the cortex. Our findings concerning the negative spike component agree with those of Pollen and Sie[7], who described the suppression of the early negative spike component after GABA application in spike-and-wave activity elicited by stimulation of the thalamus. The results are also in agreement with our report of changes in the interhemispheric cortical response under

GABA[8]. The difference between our depth recordings of unchanged polarity of the slow negative wave and those of Pollen and Sie[7] (they found positive counterpart in the 5th cortical layer) must be solved experimentally.
Our results allow us to draw the conclusion that the nature of 2 negativities, the spike and the wave components of cortical focal discharges, is different.

1 E. Roberts, T.N. Chase and D.B. Tower (eds.), GABA in Nervous System Function, p. 554. Raven Press, New York 1976.
2 E.K. Killam and K.F. Killam, in: Inhibition in the Nervous System and γ-Aminobutyric Acid, p. 527. Ed. E. Roberts. Pergamon Press, Oxford 1960.
3 B.S. Meldrum, Int. Rev. Neurobiol. *17*, 1 (1975).
4 D.B. Tower, in: GABA in Nervous System Function, p. 461. Ed. E. Roberts, T.N. Chase and D.B. Tower. Raven Press, New York 1976.
5 P. Mareš, J. Mareš, P. Remeš, M. Hessler and J. Fischer, Physiol. bohemoslov. *25*, 268 (1976).
6 P. Mareš, Brain Res. *56*, 203 (1973).
7 D.A. Pollen and P.G. Sie, Electroenceph. clin. Neurophysiol. *17*, 154 (1964).
8 P. Mareš and J. Mareš, Physiol. bohemoslov. *27*, 259 (1978).

Naloxone and intestinal motility

R.A. Howd, A. Adamovics and A. Palekar

SRI International, Life Sciences Division, Menlo Park (California 94025, USA), 6 February 1978

Summary. It was supposed that the inhibition of intestinal peristalsis seen in animals and humans after abdominal surgery might be related to the release of endorphins, endogenous opiate receptor agonists, caused by the surgical stress and pain. However, naloxone, a potent morphine and endorphin antagonist, failed to block this peristaltic inhibition in rats, which leaves the mechanism of this inhibition, and thus the function of intestinal endorphins, still very much in doubt.

There is evidence that the brain contains endogenous substances that act as agonists at morphine receptor sites[1-4]. These substances, called endorphins, are localized in specific neurons, especially along the pain pathways and the limbic system[1,3-5]. Surprisingly large quantities of endorphins are also found in the intestinal tract, especially in the longitudinal muscle layer and the myenteric plexus[2,6]. The function of endorphins in the intestinal tract is not known, but it has been shown that they are released by physical stress[3,5] or electrical stimulation in vitro[4,6-8]. Several investigators have proposed that their function in the intestinal tract is to regulate intestinal motility[8-11]. In vitro, exogenous endorphins relax or inhibit contractions of isolated intestinal segments. These and most other effects of endorphins, both in vitro and in vivo, can be blocked by the morphine antagonist naloxone[4,5,7-11]. The present study was initiated to investigate the effect of naloxone on intestinal motility in surgically stressed, conscious animals in order to determine whether the inhibition of intestinal motility seen after surgical stress might be due to release of endogenous endorphins.
Methods. Male Wistar rats (Simonsen Laboratories) were kept in a 12:12 light-dark cycle (lights on at 6.30 h). All experimental procedures began at 9.00 h. Rats to be subjected to surgical stress were anesthetized with ether, their abdomens were opened, their cecums were ligated and removed, and the wound was closed. 2 h after surgery, animals received, orally, 2 ml of a 10% charcoal meal (10% carbon black in 1% hydroxypropylmethylcellulose). 45 min later, they were killed, their small intestines were carefully removed, and the progression of the charcoal meal down the intestine was measured. This was expressed as a ratio to the total length of the small intestine, or 'percent transit'.
Control animals also received 2 ml of the charcoal suspension and were killed 45 min afterward. Naloxone in saline was administered s.c., either 5 mg/kg at the time of surgery and 2 mg/kg 20 min before the charcoal, or 0.1 or 2 mg/kg 20 min before the charcoal administration. Controls were given saline alone, at these times.
Results. The stress caused by anesthesia and cecectomy had a very dramatic effect on gastrointestinal transit of the charcoal, as shown in table 1. The s.c. administration of naloxone (2 mg/kg to unoperated animals) 20 min before the charcoal had no effect on the gastrointestinal charcoal transport.
When naloxone was administered to operated animals, no reversal of the stress-induced depression in gastrointestinal transport of charcoal occurred over a wide range of naloxone doses (0.1 mg/kg to a total of 7 mg/kg). The slight opposite tendency toward enhanced depression becomes significant ($p<0.05$) when all experiments are combined (table 2).
Discussion. No effects of naloxone on normal intestinal motility in vivo or in vitro have been observed by other workers[3,12]. Similarly, no effects were found in our experiments despite the fact that several investigators have reported a release of endorphins from the myenteric plexus under stress[5,7,9,10]. Endorphins released in this manner could be expected to inhibit intestinal motility. The administration of naloxone in these cases should then block the action of endorphins and increase intestinal motility. In guinea-pig ileum in vitro, when peristalsis is elicited by

Table 1. Effect of cecectomy or naloxone on gastrointestinal transit of a charcoal meal

Treatment	N	Transit (%)	p
Nonoperated controls	8	75.1±2.1	-
Nonoperated and naloxone (2 mg/kg)	5	75.0±3.9	NS
Operated controls	14	20.9±1.5	<0.01

Table 2. Effect of naloxone after cecectomy on gastrointestinal transit of a charcoal meal

Experiment No.	Operated controls		Naloxone treatment			p
	N	Transit (%)	N	Dose	Transit (%)	
1	4	21.3±2.7	4	2 mg/kg	15.4±0.9	<0.1
2	4	19.4±3.7	4	2 mg/kg	17.1±1.2	>0.1
			5	2 mg/kg+ 5 mg/kg	17.4±1.9	>0.1
3	6	21.7±2.2	6	0.1 mg/kg	18.9±1.7	>0.1
Overall	14	20.9±1.5	19		17.4±0.8	<0.05