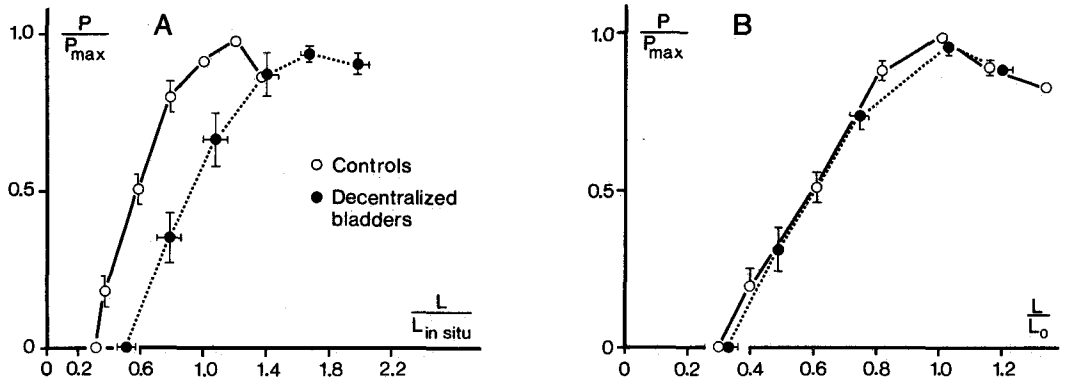


Active length-tension relations of muscle strips of decentralized bladders and those of control bladders. Abscissa: Length relative to in situ length (A); length relative to optimum length (B). Ordinates indicate force P relative to maximum force value P_{max}. The results are grouped according to $L/L_{in\,situ}$ (A) and L/L_0 (B) into classes with a width of 0.2. Bars indicate ± SE. Number of observations in each class varies between 4 and 28.

bladders were superimposable (fig. B), and there was no difference in L_{min}; i.e. L_{min}/L_0 was $0.31 \pm 0.02(5)$ for strips of control bladders and $0.33 \pm 0.03(5)$ for strips of decentralized bladders.

Tetrodotoxin added to the bath (10^{-5} g/ml) had no effect on the contractions evoked by AC stimulation, thus indicating that the muscle cells were directly stimulated.

The present study therefore shows that the decentralized bladder behaves in a similar way to the denervated bladder previously examined[4]: strips of decentralized and denervated bladders shorten less in relation to the length in situ than those of control bladders; in relation to the in situ length the strips of decentralized and denervated bladders have to be stretched to a greater extent than those of control bladders to attain the optimum length for the development of active force. Maximal active force is the same both for strips of decentralized and denervated bladders and for those of control bladders; and further, when length-tension relations are expressed in relations to optimum length there is no difference between the curves for decentralized and denervated bladders and the curve for control bladders, neither is there any difference in the ability of unloaded strips to shorten when related to optimum length.

As judged by acetylcholinesterase staining[6] and choline acetyltransferase activity[3] most cholinergic nerves persist in the urinary bladders following decentralization.

According to the parameters studied in this investigation, the presence of these nerves does not seem to influence force production of muscle cells in the hypertrophied bladder.

1 This work was supported by grants from the Swedish Medical Research Council (B82-14X-05927-02 and B82-14X-00028-18) and the Medical Faculty of Lund.
2 J. Ekström and M. Elmér, Acta physiol. scand. *101*, 58 (1977).
3 J. Ekström, Acta physiol. scand. *111*, 81 (1981).
4 J. Ekström and B. Uvelius, Acta physiol. scand. *112*, 443 (1981).
5 B. Johansson, Circulation Res. *32*, 246 (1973).
6 P. Alm and J. Ekström, Acta physiol. scand. *112*, 179 (1981).

Butanol extracts from myelin fragments: Morphological and biochemical aspects of the re-formed membranes prepared from myelin butanol extracts

R. Ishitani and K. Mizusaki[1]

Group of Biochemical Pharmacology, Josai University, Sakado, Saitama 350-02 (Japan), 11 November 1981

Summary. The re-formed membranes prepared from butanol extracts of myelin were examined by morphological and biochemical methods. Using freeze-fracturing, re-formed membranes showed 2 types of assembly of membrane particles, i.e., myelin-like and cluster arrangements. Moreover, SDS-urea disc gel electrophoresis indicated that the protein composition of these membranes reflected that of the myelin fragments.

For the isolation of receptor components from biological tissues, an organic solvent extraction technique has been employed to isolate the proteolipid-like receptors[2-5], based on the assumption that the receptor components present in the biological membranes are closely associated with lipids. However, the question has been raised whether this method preserves the inherent biophysical nature of receptor components. Using negative staining for electron microscopy, Vásquez et al.[6] investigated the morphological features of the chloroform-methanol extracts from bovine and cat cerebral cortex, but their biophysical properties were not examined. The objective of the present work was to examine to what extent butanol extracts of myelin (proteolipids), which have a specific binding capacity for 5-hydroxytryptamine (5-HT)[7-9], retain their original biophysical properties. Freeze-fracturing reveals the hydrophobic central plane of biological membranes upon which the lipid-protein interactions are reflected in the occurrence of distinct particles, i.e., membrane particles[10]. Since such interaction may be a criterion for examining the biophysi-

cal property of the proteolipid macromolecules isolated from biological membranes, we used this method as well as biochemical analyses.

Materials and methods. The details of preparation of myelin butanol extracts were as reported previously[8]. Briefly, the myelin fragments were isolated from rat brain stems by the method of Norton and Poduslo[11] and extracted with water-saturated butanol. These extracts were dialyzed for 48 h at 4 °C against 500 volumes of 0.1 M phosphate buffer (pH 7.3) with 3 changes at intervals of 12 h. The re-formed membranes were obtained as quite thin artificial membranes and total recoveries both of protein and of lipid phosphorus from the myelin butanol extracts were 95% or over. For freeze-fracturing, the prefixed reformed membranes in 4% glutaraldehyde were impregnated with 30% glycerol. The fixed samples were mounted in metal cups, quickly frozen in liquid Freon 22 and set on the specimen table of an EIKO FD-2A freeze-fracture apparatus. Fractures were made at -120 °C under 10^{-6} Torr and shadowed with platinum-carbon under rotation. The experimental details of SDS-urea disc gel electrophoresis were as described previously[12]. The butanol suspension of re-formed membranes was also treated with water as well as the myelin butanol extracts (14%, v/v), and aliquots of each sample (approximately 2 mg protein/1.5 ml) were incubated for 20 min with 5×10^{-7} M of $^{14}C\cdot$5-HT. After incubation, the mixtures were loaded on to a Sephadex LH_{20} column (1.3×20 cm). Stepwise elution was carried out with solvents of increasing polarity, and radioactivity was counted as described previously[8].

Results and discussion. Pinto da Silva and Miller[13] indicated the constant and widespread presence of membrane particles on the fracture faces of myelin, and they proposed that

Table 1. Protein compositions of several preparations

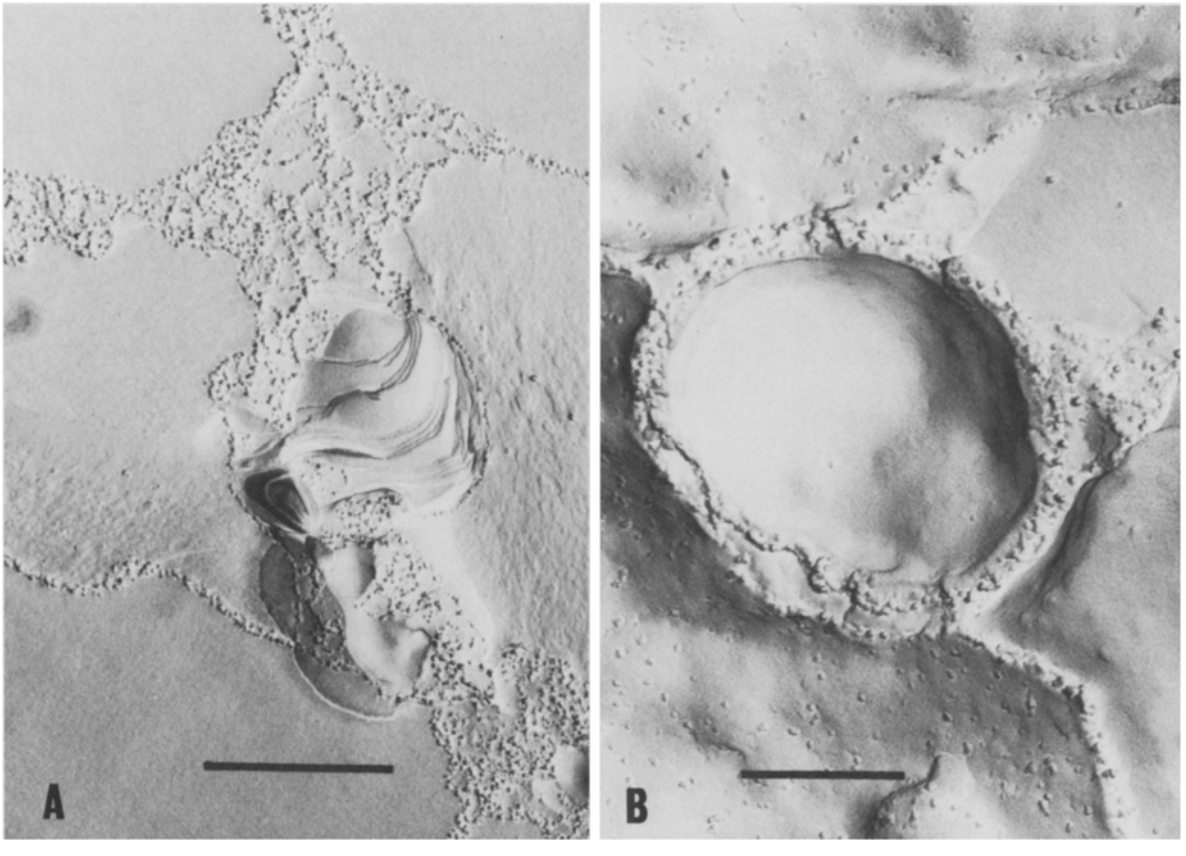
Protein	Myelin fragments	Myelin butanol extracts	Re-formed membranes
HMW	27.6±1.5	13.2±0.6	28.4±2.0
PLP	15.0±0.7	20.8±1.2	14.0±1.1
DM-20	3.2±0.4	3.4±0.3	3.3±0.3
BPs	47.0±1.3	55.2±1.2	46.9±1.5
(Rest)	7.2±0.3	7.4±0.2	7.4±0.5

Methods of gel electrophoresis were described previously[12]; 75 µg protein of each sample was loaded on to a 8.5% gel. Results are expressed as percentage of the dye binding capacity (mean ± SEM of 5 experiments). HMW, high molecular weight proteins; PLP, proteolipid protein; and BPs, basic proteins.

Table 2. $^{14}C\cdot$5-HT binding to myelin butanol extracts and re-formed membranes

Sample	Amount of 5-HT bound (%)
Myelin butanol extracts	40.3±2.0
Re-formed membranes	40.7±1.9

After incubation of samples with 5×10^{-7} M of $^{14}C\cdot$5-HT, the mixtures were chromatographed through a Sephadex LH_{20} column and the specific bound radioactivity was measured as reported previously[8]. Results are expressed as the percent of input total radioactivity (mean ± SEM of 4 experiments).



Freeze-fracture electronmicrographs of the re-formed membranes prepared from myelin butanol extracts. *A* Multilamellar structure and clusters of membrane particles can be seen. Bar equals 0.5 µm. *B* A myelin-like arrangement of membrane particles is observed. Bar equals 0.25 µm.

these membrane particles probably represent proteolipid protein present in the membranes. In addition, it is plausible to presume that some substances of high molecular weight proteins may also be implicated in the constitution of these particles. Freeze-fracture electronmicrographs of the re-formed membranes (figs A and B) showed the 2 types of assemblies of membrane particles. One is the myelin-like arrangement and this type was also recognized on the fracture faces of myelin. The other is a cluster-arrangement and for the occurrence of this type of assembly, the explanation is obscure. However, it seems likely that the intrinsic protein(s) showing hydrophobicity more actively interact with each other under the hydrophilic conditions which were applied in order to prepare the re-formed membranes, and thus the formation of cluster-arrangements is induced. The occurrence of clusters was more frequent than that of the other arrangements.

To obtain more detailed information on the molecular constituents of re-formed membranes, protein composition was analyzed (table 1). An interesting finding was that the protein composition of these membranes closely corresponded to that of myelin. When the re-formed membranes are formed from myelin extracts, it is still more likely that each of the components dissolving in organic solvents may be re-organized according to their mutual biophysical properties. Thus, this evidence supports the idea that the molecules of myelin proteolipids maintain their inherent biophysical characteristics well. Moreover, $^{14}C \cdot 5$-HT binding experiments were performed to examine whether or not the re-formed membranes possess a 5-HT binding capacity. The results (table 2) indicated that these membranes also had the same 5-HT binding capacity as the original myelin extracts. Although studies on the identification of 5-HT binding components from myelin proteolipids are not yet complete, we have reported that other component(s) besides lipids may be implicated in the binding of 5-HT[12,14].

Finally, these results suggest that the organic solvent extraction method retains the inherent biophysical properties of the molecules present in myelin membranes satisfactorily, and thus this technique may provide a tool for studying the nature of receptor or binding components which are strongly associating with lipids in biological membranes.

1 Acknowledgment. This work was supported by a research grant (No. 467384) from the Ministry of Education, Japan.
2 S. Fiszer De Plazas and E. De Robertis, J. Neurochem. *25*, 547 (1975).
3 S.F.R. Godwin and J.M. Sneddon, J. Neurochem. *25*, 283 (1975).
4 R.F. Taylor, J. Neurochem. *31*, 1183 (1978).
5 B.D. Boyan-Salyers and Y. Clement-Cormier, Biochim. biophys. Acta *617*, 274 (1980).
6 C. Vásquez, F.J. Barrantes, J.L. La Torre and E. De Robertis, J. molec. Biol. *52*, 221 (1970).
7 R. Ishitani, A. Miyakawa, R. Saito and T. Iwamoto, Experientia *33*, 932 (1977).
8 R. Ishitani, A. Miyakawa and T. Iwamoto, Jap. J. Pharmac. *28*, 899 (1978).
9 R. Ishitani, Y. Tobari, A. Miyakawa and T. Iwamoto, Experientia *34*, 1628 (1978).
10 P. Pinto da Silva and D. Branton, J. Cell Biol. *45*, 598 (1970).
11 W.T. Norton and S.E. Poduslo, J. Neurochem. *21*, 749 (1973).
12 R. Ishitani and A. Miyakawa, Experientia *34*, 1605 (1978).
13 P. Pinto da Silva and R.G. Miller, Proc. natl Acad. Sci. USA *72*, 4046 (1975).
14 R. Ishitani, A. Karasawa and A. Miyakawa, Life Sci. *27*, 291 (1980).

Reinforcement with naloxone of N-n-propylnorapomorphine (NPA) capability for stimulating male rat copulatory behavior

Francesca Ferrari and G. Baggio

Institute of Pharmacology, Chair of Pharmacology and Pharmacognosy, University of Modena, Via G. Campi 287, I-41100 Modena (Italy), 20 November 1981

Summary. Naloxone at a dose of 2 mg · kg^{-1}, which per se did not significantly alter the copulatory pattern of sexually active adult male rats, did significantly reduce the intromission frequency as well as latency to ejaculation when administered before a low and inactive dose of N-n-propylnorapomorphine (0.5 µg · kg^{-1}).

Recent studies suggest that naloxone (Nx), widely considered to be an opiate antagonist, may possess a stimulant effect on sexual behavior[1,2]. Moreover the administration of Nx is reported to potentiate certain effects of dopamine-mimicking drugs[3-5].

Previous research in our laboratory established that low N-n-propylnorapomorphine (NPA) doses, i.p. injected into adult male rats, caused a significant increase in the number of spontaneous episodes of penile erection (PE) during a 1-h observation period, presumably through activation of specific DA-receptors in the CNS[6,7]. Nx (0.5–4 mg · kg^{-1}) which per se caused only a modest increase in PE, greatly potentiated the effect of NPA, and the maximum response to NPA 8 µg · kg^{-1} was far in excess of that produced by the most effective NPA dose[7].

The present experiments were carried out to ascertain how the combination of Nx and NPA alters the copulatory behavior of the adult male rat.

Methods and materials. Adult male Wistar rats (S. Morini, S. Polo d'Enza, Reggio Emilia, Italy), initial weight 230 g, were employed. They were housed in groups of 5 and maintained on ad libitum food and water in a quiet climatized (22 ± 1 °C, 60% humidity) room under a reverse light-dark cycle (with light from 23.00 h to 11.00 h).

Female rats (initial b.wt 230 g) of the same strain used as mating stimulus were brought into estrus with a s.c. injection of 0.12 mg estradiol benzoate 48–72 h before use[8]. The tests were performed during the early part of the dark phase. Male copulatory behavior was evaluated as by Dewsbury[9] and the following were recorded: mount and intromission latencies (ML and IL) (time elapsed from the introduction of the female into the cage until the 1st mount and intromission, respectively), mount and intromission frequency (MF and IF) (number of mounts and intromissions preceding ejaculation), ejaculation latency (EL) (interval from the 1st intromission to ejaculation), post-ejaculatory interval (PEi) (time from the 1st ejaculation to the next 1st intromission).

Tests were discontinued when IL or PEi were > 15 min or EL was > 30 min. Of 94 male rats at the start of the