Table 1. Distribution of radioactivity within regions of the fast axon branches and terminal in locust extensor tibiae muscles stimulated at 0.5 Hz and rested in radiolabelled L-glutamate for 1 h

Source of radioactivity	Radioactivity/unit area grains/μm^2 n = 30	SE
Axon branch		
Muscle fibre	0.087	0.019
Axon branch (axoplasm)	0.000	0.000
Glial wrappings	0.298	0.105
External	0.182	0.054
Axon terminal		
Muscle fibre	0.019	0.011
Axon terminal (axoplasm)	0.000	0.000
Glial cell	4.007	0.965
External	0.056	0.051

Table 2. Distribution of radioactivity within regions of the fast axon branches and terminals in locust extensor tibiae muscles stimulated at 100 Hz and rested in radiolabelled L-glutamate for 1 h

Source of radioactivity	Radioactivity/unit area grains/μm^2 n = 30	SE
Axon branch		
Muscle fibre	0.207	0.070
Axon branch (axoplasm)	0.076	0.159
Glial wrappings	0.964	0.256
External	0.555	0.250
Axon terminal		
Muscle fibre	0.223	0.025
Axon terminal (axoplasm)	1.848	0.364
Glial cell	2.796	0.806
External	0.194	0.036

SE refers to the variation in the counting procedure in the analyses. External regions include, basement membrane, teacheoles and haemolymph space. Axon terminal resp. (axoplasm) includes axoplasm + organelles.

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present study was to examine the uptake of exogenously applied radiolabelled L-glutamate at 'normal' and depleted excitatory neuromuscular junctions.

Materials and methods. Isolated extensor tibiae nerve-muscle preparations were stimulated and prepared for electron microscopy as described previously[4], except that following stimulation the preparations were incubated in saline containing L-[G-^{3}H]glutamic acid (final concentration 4×10^{-6}M and 125 μCi per ml) or DL-[4,5-^{3}H]-leucine (final concentration 2×10^{-5}M and 20 μCi per ml). Thin sections (pale gold), coated with Ilford L-4-emulsion using a variation of the loop technique of Caro and Van Tubergen[5], were exposed for 20 weeks at 4 °C and developed in Microdol X. Sections were examined in an AEI EM 6B electron microscope and the distribution of silver grains in the electron autoradiographs analyzed using the method described by Blackett and Parry[6]. This method takes into account the cross scatter of grains between neighbouring structures for the actual shapes occurring within the section and no assumptions are required about the size, shape or spatial arrangements of these structures[7].

Results and discussion. Radiochemical experiments indicated that unstimulated whole muscle preparations had a high affinity for L-glutamate relative to L-leucine which was used as a control. Furthermore, fatigued preparations prestimulated at 100 Hz showed an increased uptake of L-glutamate compared to unstimulated control preparations, while there was no change in the uptake of L-leucine. Control muscles incubated in ^{3}H L-glutamate and prepared for light microscope autoradiography indicated that the radioactivity was distributed uniformly over the sections while sections from stimulated muscles displayed 'hot spots' over regions at the muscle fibre surfaces presumed to be sites of nerve endings.

Analysis of the grain distribution in preparations stimulated at 0.5 Hz revealed that the radioactivity was mainly associated with the glial wrappings at the fast axon branches and glial cells at the axon terminals while there was little, if any, radioactivity accumulated by the axoplasm (table 1). This confirms the work of earlier investigators[8,9] who have suggested that the glial cells which cap the terminals play a major role in glutamate uptake at axon terminals and thus provide a means of inactivating the transmitter. In preparations stimulated at 100 Hz to the point of fatigue, radioactivity was again sequestered by the glial cells but in addition substantial radioactivity was found to be present in the axoplasm of the axon terminals (table 2, figure).

These results are considered to support the hypothesis that L-glutamate is the transmitter at the excitatory neuromuscular junctions of the locust extensor tibiae muscle.

Correlation between acidic phospholipids and serotonin and between lysolecithin and dopamine in ganglia of the marine mussel, *Mytilus edulis*[1]

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Summary. These studies have demonstrated a positive correlation between the acidic phospholipids and the serotonin content and between the lysolecithin and the dopamine content in the cerebral, pedal and visceral ganglia of *Mytilus edulis*. These relationships were further supported by experiments utilizing 6-hydroxydopamine and 5,6-dihydroxytryptamine.

Several recent metabolic studies[5-9] and reviews[10,11] have implicated a functional relationship between neurotransmitters and phospholipids. Hokin[12] and Hokin[13] have generalized this relationship by suggesting that excitatory transmission with acetylcholine, norepinephrine and serotonin (5-HT) results in an increase in the labeling of phosphatidate and phosphatidylinositol; while inhibitory transmissions such as that generally seen with

γ-amino butyric acid (GABA) and dopamine (DA), involves a decrease in labeling. These relationships suggested that the phospholipid content of nervous tissue might reflect the amounts of monoamines present. Thus, it was the objective of this study to determine a quantitative relationship between specific phospholipids and monoamines in the ganglia of *Mytilus edulis*. Secondly, since previous studies[14] have shown that the 5-HT and DA content of these ganglia are modified by 6-hydroxydopamine (6-OHDA) and 5,6-dihydroxytryptamine (5,6-DHT), these drugs were used to detect concomitant changes in the amount of specific phospholipids.

Materials and methods. The marine mussel, *Mytilus edulis*, were collected and maintained as described previously[14]. The cerebral (CG), pedal (PG), and visceral (VG) ganglia were removed, extracted and separated using a combination of chromatographies with DEAE-Sephadex A-25[15] and TLC of the separated neutral or zwitterionic lipids (F_1) and acidic lipids (F_2) on silica gel HR precoated plates using a solvent system of chloroform/methanol/15M NH_4OH (70/30/5 by volume). The bands corresponding to authentic standards were scrapped off and analyzed for phosphorus[16] which was assumed to represent an average of 4% of the phospholipid weight. In another series of experiments, the aforementioned ganglia from 4 animals were separately collected, pooled and quantified for 5-HT and DA spectrofluorometrically[17,18] and the data confirmed previous histofluorescent observations[14,19]. In the drug treated animals, 6 days prior to the removal of the ganglia, 100 µg of 6-OHDA or 5,6-DHT, was prepared and injected with appropriate controls as previously described[14,20].

Results and discussion. The ganglia were found (table 1) to contain membrane associated phospholipids (PL): sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and the acidic phospholipids (F_2) which included largely phosphatidylinositols and phosphatidylserine. In addition, a band co-migrating with lysolecithin (lyso PC) was noted and amounted to about 7% of the total. Some interesting parallels became apparent between the amounts of lyso PC and DA among the various ganglia; for example, the VG which contained the greatest content of lyso PC also had the largest amount of DA. This relationship showed a correlation coefficient (r) of 0.92 among the examined ganglia. Similarly, the 5-HT content paralleled the amount of acidic phospholipids with the greatest amounts seen in the CG. The poorer correlation (0.74) seen for the acidic PL and 5-HT may be partially attributed to the multiple components found in this lipid fraction. However, if a comparison is made between the ratios of acidic PL/lyso PC to the corresponding monoamine ration (5-HT/DA) as shown in table 1 a much more convincing correlation was found suggesting that the interrelationships between 5-HT and DA are also reflected in the relative association of acidic PL with lyso PC. In addition, 5-HT showed a similar positive correlation with the other phospholipids, except with lyso PC. This probably reflected the substantially larger content of 5-HT than DA in these

Table 1. Content and correlation of phospholipids with monoamines of the cerebral (CG), pedal (PG) and visceral (VG) ganglia

Component	CG	PG	VG	+ r
SM (mg/g)	2.04	1.47	1.07	0.874
PC (mg/g)	2.43	1.82	1.57	0.800
PE (mg/g)	3.18	1.91	1.67	0.709
Unknowns (mg/g)	1.25	0.98	0.94	0.688
Acidic PL (mg/g)	2.07	1.38	1.21	0.736
5-HT (µg/g)	28.79 + 0.94	27.98 ± 1.89	22.4 + 1.38	
Lyso PC (mg/g)	0.50	0.52	0.81	0.923
DA (µg/g)	7.9 ± 0.48	11.63 ± 0.86	16.4 ± 0.70	
Acidic PL/Lyso PC	4.14	2.65	1.49	1.000
5-HT/DA	3.64	2.41	1.37	

The phospholipids represent an average of 2 independent determinations which agreed to within 80–88%. The corresponding monoamine is grouped with its correlated lipid(s) and they are expressed as the mean ± SEM (n = 4). The bottom group shows the ratios of the correlated components of interest. The positive correlation coefficient, + r, is defined as MO_x/O_y where M = slope of the specific phospholipid to the corresponding monoamine relationship, $O_x = \sqrt{\text{phospholipid variance}}$ and $O_y = \sqrt{\text{monoamine variance}}$.

Table 2. Phospholipid content of ganglia treated with drugs which modify the serotonin and dopamine content

Component	5,6-DHT treated % of control CG	PG	VG	6-OHDA treated % of control CG	PG	VG
Lyso PC	38.9	75.3	109.2	24.2	48.3	55.0
SM	101.8	101.3	96.8	89.5	78.8	44.9
PC	92.1	99.1	99.6	89.5	100.0	92.7
PE	85.2	95.0	91.3	92.6	97.3	86.2
Acidic PL	56.9	68.1	88.6	138.6	189.4	142.0
Total PL	84.9	95.2	97.5	82.2	87.5	74.8

The % changes represent the average of 2 independent determinations which agreed to within 90%. The cerebral, pedal and visceral ganglia are designated CG, PG and VG respectively.

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ganglia. These highly suggestive correlations were further supported by pharmacological manipulation (table 2). 5,6-DHT, which destroys 5-HT neurons[20] in the ganglia has a concomitant effect on the acidic PL, especially in the rich 5-HT containing CG. The minimal changes seen in the VG parallels its relatively smaller content of 5-HT. Furthermore, our previous studies[14] have shown a reciprocal relationship between DA and 5-HT but it is possible that in the reverse situation, i.e., 5-HT effects on DA, a more directly proportional relationship may exist as suggested by the 5,6-DHT effects on lyso PC (table 2). The destruction of dopaminergic neurons by 6-OHDA produced an increase (about 73% in the pedal ganglia[14]) in the 5-HT content. These previously reported changes[14] paralleled the PL changes (table 2) in that there was a marked decrease in lyso PC matched with a significant increase in the acidic PL.

The data presented suggests a functional relationship between specific phospholipids and monoamines in ganglia of *Mytilus edulis*. More direct evidence such as that shown for the stimulation of enzymes of monoamine metabolism by specific phospholipids[21] are needed to confirm these suggestions. Portions of this work have been presented previously[22].

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Effect of electroconvulsive treatment on serum dopamine-beta-hydroxylase activity in man

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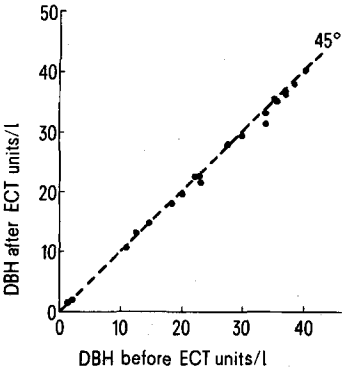
Summary. The activity of dopamine-beta-hydroxylase was measured in the serum before and immediately after electroconvulsive treatments. No significant difference was observed, suggesting that the seizures did not cause an increase in the peripheral sympathetic tone.

Effective electroconvulsive treatment (ECT) causes major convulsions and thus can be clinically and biochemically paralleled with epileptic major convulsions. Spontaneous grand-mal seizures are accompanied by autonomic changes but direct evidence of sympathetic activation is meagre. Heart rate and blood pressure are slightly changed but these changes may be secondary to other changes (e.g. respiratory). ECT, like spontaneous grand mal seizure, is believed to cause a simultaneous discharge of (all?) brain neurons. ECT was found to increase serum prolactin level[3]. Ohman et al.[3] discuss the possibility that the effect on serum prolactin is due to a general nonspecific CNS activation, and since ECT was not found to cause a significant increase of thyroid stimulating hormone[4], they concluded that the effect of ECT was more or less specific. There is evidence that ECT activates central adrenergic mechanisms[4]. The present study was carried out to determine whether sympathetic activation occurs in the periphery in humans. The levels of dopamine-beta-hydroxylase (DBH) in serum before and after ECT were used as a measure of peripheral sympathetic activity[5]. This enzyme is responsible for the last step in the synthesis of noradrenaline with which it is released from sympathetic nerve endings.



Serum DBH activity following ECT plotted against pre-ECT activity. Each point represents the activity values preceding and following a single treatment.

Materials and methods. Patients. The study included 8 patients, 6 males and 2 females, ranging in age from 14 to 65 years. 7 patients were diagnosed as schizophrenic and 1 as involutional depressive. Each patient underwent several ECT treatments and DBH was examined in 2 or 3 of these in each patient. 2 of the patients were not receiving drugs and the others were on antipsychotic (haloperidol, fluphenazine or chlorpromazine) or antidepressant (imipramine or chlorimipramine) drugs.

ECT procedure. Following bed-rest for 30 min, the patient was premedicated by thiopental, 4 mg/kg, and succinylcholine, 0.5 mg/kg i.v. Electroshock treatment was immediately delivered by a Siemens Konvulsator 2077 with continuous pulse sequence at an intensity of 400–550 mA peaked current referred to a patient resistance of 300 ohms and current flow duration of 0.8–1.6 sec. In all patients at least a minor seizure was observed in response to the ECT.

Enzyme assay. Venous blood samples were collected from the patients before premedication and once again 30–150 min following the ECT, while the patients were still prone. The samples were allowed to clot at room temperature, and the serum was separated by centrifugation and stored at −5 °C until assayed. The activity of DBH in serum was measured according to Nagatsu and

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