Distribution of radioactivity after administration of ^{3}H-5-hydroxytryptamine by three different routes to the mussel *Mytilus edulis*

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Summary. Intracardiac injection gives proportionately higher and earlier peak concentration in tissues that specifically accumulates it, i.m. injection gives slower but longer lasting accumulations, and topical application to a ganglia essentially localizes the drug in that structure.

Various authors have given pharmacological agents to the mussel *Mytilus edulis* in an attempt to uncover monoaminergic mechanisms. These agents have been injected into the visceral mass[3] and into the posterior addustor muscle[4-6], applied topically to a specific ganglion[7] and added to the medium bathing whole mussels[8] and isolated parts[9,10]. The actual site or sites of action of the agents has generally not been unequivically demonstrated, especially in the whole organism, where the question of central versus peripheral locus of action remains unanswered. The purpose of the present study is to examine the distribution of ^{3}H-5-hydroxytryptamine (5-HT) after its administration to the whole organism by 3 different routes with a view towards delivering the drug more selectively to specific structures.

Materials and methods. M. edulis was acquired and maintained as previously described[11]. Administration of 15 ng of ^{3}H-5-HT (New England Nuclear, 700,000 dpm) in 10 µl of filtered sea water was made with a 10 µl Hamilton Syringe topically to the pedal ganglia, into the posterior adductor muscle and into the heart. I.m. injection was done as previously described[5]. Application to the pedal ganglia and into the heart was made over a 20-min period, giving 1 µl every 2 min. The pedal ganglia were reached through the ventral shell opening after separating the sheet of tissue overlying them at the base of the foot between the anterior byssus retractor muscles (ABRM). The heart was reached through a hole drilled in the hinge above the heart. The needle was held at a sharp angle to facilitate penetration of the ventricle while avoiding the rectum. During the period of injection the needle was supported by putty and the syringe barrel by a micromanipulator. After all injections the animals were rinsed briefly and each placed in 100 ml of fresh medium. For counting, whole ganglia (about 2 mg) and small portions of tissue (about 50 mg) from the center of 1 gill, mantle and ABRM were removed and dissolved in Protosol (New England Nuclear). Scintillation counting was done in a Packard Tricarb using a medium containing 5.5 g Permablend III in 1L Toluene Puresolv (Packard). Data are expressed as dpm/mg wet weight, correcting for quenching by the use of internal standards and for background. Some experiments were performed with non-labeled 5-HT and the tissue extracted and assayed for 5-HT fluorometrically as previously described[7].

Results and discussion. The data, summarized in the table, shows that the greatest concentration of radioactivity following i.m. and intracardiac administration occurs in the ganglia constituting the central nervous system. High concentrations in the gill may reflect the serotonergic innervation of that organ[3,11] and its ability to synthesize and store 5-HT[7,12]. The low concentrations in the mantle represents the lack of serotonergic elements and therefore, nonspecific accumulations. Accumulations in specific tissues known to contain serotonergic structures reached their peaks more rapidly after intracardiac injection. The muscle appeared to act as a reservoir for the injected material, slowly releasing it to the rest of the body, and unpublished histofluorescent photomicrographs showed considerable nonspecific binding of 5-HT to the muscle tissue after 48 h. The close proximity of the visceral ganglia to the injected muscle probably accounts for faster accumulation of labeled material after i.m., compared to intracardiac injection.

H-5-HT distribution

	^{3}H-5-HT (dpm/mg ± SEM)					
	30 min	1 h	2 h	6 h	12 h	24 h
ıtramuscular						
g	116.36± 34.53	372.93± 41.33	735.6 ± 78.67	3080 ±198.67	2498.66±226.66	710.40± 89.33
g	142.93± 34.67	512.93± 60	856.4 ± 21.33	5121.33±272	4234.66±118.66	1428.93±152.00
g	440.13± 61.33	932.93± 94.67	2589.47±270.67	6374.67±454.67	5169.33±245.33	2984.00±285.33
BRM	38.27± 13.33	72.4± 24	188.8 ± 49.33	250.27± 60	226.66± 85.33	125.73± 17.33
ill	63.20± 28	325.07± 28	392.67± 92	512.13±149.33	689.33± 85.33	253.00± 36.00
lantle	14.00± 4.93	20± 6.67	36± 9.87	40.53± 21.33	32.00± 10.66	17.33± 5.33
ıtracardiac						
g	369.06±112.00	8428.00±416.00	7262.66±557.33	2821.33±153.33	1476.00±286.66	974.66±228.00
g	575.73± 85.20	10832.00±680.00	8378.66±281.33	4453.33±378.66	1909.33±424.00	1302.66±218.66
g	515.46± 50.13	11094.66±584.00	9257.33±717.33	3858.66±416.00	2189.33±318.66	1198.66± 88.00
BRM	164.93± 24.00	238.66± 52.00	217.33± 32.00	165.33± 53.00	174.66± 45.33	134.66± 12.53
ill	424.00± 70.66	781.33± 84.00	644.00± 68.00	284.00± 57.33	229.33± 34.66	190.66± 38.66
lantle	16.00± 10.66	42.93± 12.00	34.66± 13.33	18.00± 12.00	15.33± 11.33	12.00± 6.66
opical to pedal ganglia						
g	45.33±41.33	33.33± 17.33	32.00± 13.33	18.66± 9.33	13.33± 9.33	10.66± 9.33
g	250.030.66±13.841.33	231.730.66± 13.125.33	201.762.66± 13.988.00	115.921.33± 10.358.66	55.036.00± 8.578.66	42.212.00± 3.081.33
g	58.66± 32.00	48.00± 41.33	50.66± 18.66	40.00± 20.00	42.66±24.00	34.66± 8.00
BRM	32.13± 14.66	28.00± 18.66	24.00± 8.00	34.66± 10.66	49.33±13.33	76.00± 10.66*
ill	-	20.00± 10.66	-	28.00± 21.33	45.33±25.33	48.00± 20.00
lantle	-	-	14.66± 13.33	17.33± 6.66	-	13.33± 5.33

ata is expressed as dpm (corrected for quenching and background). Each reading constitutes the mean taken from 4 animals ± SEM. Cg, erebral ganglia; Pg, pedal ganglia: Vg, visceral ganglia; ABRM, anterior byssus retractor muscle. The one tailed Student's t-test was employed or the test of significance. * $p < 0.01$ as compared to 2 h reading.

Topical application to the pedal ganglia resulted in an immediate high local concentration with slow and minimal distribution to other tissues.

We believe that within the 1st 30 min, and probably the 1st day, the total radioactivity represents mainly 5-HT and not its metabolites for the following reasons. Nonlabeled 5-HT was applied to the pedal ganglia of 8 mussels and the ganglia extirpated after 30 min from 4 and after 24 h from the other 4, extracted and assayed fluorimetrically for 5-HT. After subtracting the control value for 4 untreated mussels (23–27 ng) it was found that 32–40% of the original 1 µg dose was retained after 30 min and 4–7% after 24 h. The approximate 8fold decrease in 5-HT corresponds roughly to the 6fold decrease in total radioactivity retained by the ganglion over the same time span and suggests that at least 75% of the label represents 5-HT. Fluorescence analysis of 5-HT in all 3 ganglia was also measured after intracardiac injection of nonlabeled 5-HT and again, the relative distribution and change with time in the amount found compared favorably with the total radioactivity data. The rate of metabolic degredation of endogenous 5-HT is unknown although Stefano and Aiello[4] found that inhibition of monoamine oxidase enhanced histofluorescence within 24 h.

The increase in radioactivity in the ABRM, innervated by 5-HT fibres[13], between 2 and 24 h can be accounted for by axonal transport from the pedal ganglia at a rate of 42.2 mm/days as calculated by the method of Dahlström[14]. Experiments in progress suggest that most declines in radioactivity are due to excretion into the bathing medium with insignificant reabsorption. The amount of 5-HT estimated to be accumulated by various tissue is well within physiological limits and is presumably being metabolized normally. For example, the 2 h reading for the visceral ganglia after i.m. injection represents less than 2% of the estimated content and that in the pedal ganglia 30 min after topical administration represents less than 20% of the content, calculated from present and previous data[15]. In summary, intracardiac injection gives proportionately higher and earlier peak concentrations in tissues that specifically accumulate it, i.m. injection gives slower but longer lasting accumulations, and topical application to a ganglion essentially localizes the drug in that structure.

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Changes in cholinesterase activity of muscle after crushing the sciatic nerve of rats[1]

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Summary. The subcellular distribution of cholinesterase (ChE) was studied in the gastrocnemius muscle of rats after strong or weak nerve crushing. The ChE activities of muscle were decreased to a greater extent by strong crushing than by weak crushing. In particular, the ChE activity of the fraction containing sarcoplasmic reticulum was most greatly decreased. These results suggest that the change in the ChE activity of the microsomal fraction most finely reflects the strength of nerve crushing.

It is well known that the ChE activity of muscle decreases after denervation. Many authors, however, have reported the changes in the level of homogenate[2,4] and histochemical staining[3–5]. It has been reported that under normal conditions ChE was distributed on the sarcolemma membrane, and to a considerable extent on the sarcoplasmic reticulum[6–8]. Therefore, this paper describes the distribution of the ChE activity in rat gastrocnemius muscle after strong or weak nerve crushing.

Methods. Male Wistar-Imamichi rats weighing 110–130 g were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). The left sciatic nerve was crushed over a length of 2 mm under constant pressure at the level of the thigh for 5 min with Péan's forceps whose contact surfaces had been flattened. The details of the method of crushing the nerve will be described elsewhere[9]. The contralateral muscle served as a control. The muscle was prepared as follows. The gastrocnemius muscle was rapidly removed, minced at 0 °C and homogenized in 0.6 M KCl-10 mM Tris-maleate buffer (pH 7.4). After incubation at 37 °C for 30 min, the homogenate (3.2%, w/v) was centrifuged at 1,000 × g for 5 min. The resulting precipitate was called fraction *a*. The supernatant was diluted 4fold with distilled water, and centrifuged at 100,000 × g for 60 min. The resulting precipitate was called fraction *b*, and the supernatant, fraction *c*. Fraction *a* and *b* was suspended in modified Krebs Ringer's solution (final Ca^{2+} concentration, 1 mM). No solution was added to fraction *c*. Fraction *a*, *b* and *c* contained mainly sarcolemma ChE, sarcoplasmic reticulum ChE and solubilized ChE, respectively. On the following day the ChE assay was performed. The ChE activity, determined by the slightly modified method of Chuang[10], was calculated from the ^{3}H-acetate extracted into toluene-isoamylalcohol (5:1, v/v) produced from ^{3}H-acetylcholine hydrolyzed in 10 min. 1 unit is defined as the hydrolyctic activity which will produce 10^{-9} moles of acetylcholine per 1 min. Protein concentrations were estimated by the method of Lowry et al.[11].