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SELECTIVE DESTRUCTION OF 5-HYDROXYTRYPTAMINE RECEPTORS BY NEURAMINIDASE

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Summary

The responses of isolated frog skin to 5-hydroxytryptamine (increased active sodium transport and decreased passive chloride permeability) are diminished by incubation with the enzymes neuraminidase and *N*-acetylneuraminic acid aldolase but only in the absence of Ca^{2+} and presence of EDTA. The responses induced by oxytocin, adrenalin and aldosterone are unaffected by enzyme treatment.

The short circuit current (I_{sc}) and transepithelial resistance across isolated frog skin increase on the addition of 5-hydroxytryptamine to the serosal medium [1]. It has been shown that the increase in I_{sc} is equal to the increase in active sodium transport and the total increase in resistance is equal to the decrease in the passive chloride permeability in the mucosal-serosal direction and that both these effects are mediated via a single 5-hydroxytryptamine receptor type which is distinct from the neurohypophyseal octapeptide (oxytocin) receptor and α- and β-adrenoceptors [1, 2]. There is evidence that the 5-hydroxytryptamine receptor in smooth muscle is closely linked to membrane sialic acids (acylated neuraminic acids) [3, 4]; in this work the effect of neuraminidase, which catalyses the removal of sialic acids from glycolipids and glycoproteins, was studied together with the effects of *N*-acetylneuraminic acid aldolase (Acneu-aldolase) which splits *N*-acetylneuraminic acid (AcNeu) into *N*-acetyl-D-mannosamine and pyruvate on the 5-hydroxytryptamine, oxytocin, adrenalin and aldosterone responses of frog skin.

Frogs, *Rana temporaria*, were rapidly pithed. The ventral skin was removed, stretched across a double Ussing chamber and incubated in aerated Ringer solution (compositon, mequiv./l: Na^+ 113.5; K^+ 3.5; Cl^- 116.5; HCO_3^-

2.4; Ca^{2+} 0.89; pH 7.8) for 60 min. In the first series of experiments (Table I) the chambers were drained and the skin in one half chamber incubated in normal Ringer (control) and the other in either normal Ringer or Ca^{2+}-free Ringer containing 1 mM EDTA; after 30 min the enzyme (0.1 IU neuraminidase; 0.15 IU AcNeu-aldolase; Sigma Co.) was added to the serosal side of the experimental half chamber and incubated for 2 h. Each half chamber was then rinsed three times with normal Ringer, normal Ringer was added to the mucosal side and normal Ringer containing 10^{-5} M 5-hydroxytryptamine (creatinine sulphate complex; Sigma Co.) added to the serosal side of both half chambers. A second series of experiments (Table II) were performed fol-

TABLE I

THE EFFECT OF NEURAMINIDASE AND AcNeu-ALDOLASE ON THE I_{sc} AND TRANSEPITHELIAL RESISTANCE RESPONSES OF FROG SKIN TO THE SEROSAL ADDITON OF 10^{-5} M 5-HYDROXYTRYPTAMINE

Ventral skin was incubated in either normal Ringer or Ca^{2+}-free Ringer + 1 mM EDTA. Enzyme (0.1 IU neuraminidase, 0.15 IU AcNeu-aldolase) was added to the serosal medium and the tissue incubated for 2 h. Normal Ringer was then added to the mucosal side and normal Ringer + 10^{-5} M 5-hydroxytryptamine added to the serosal side. Responses are calculated as the maximum % increase in I_{sc}/resistance ± S.E.M. (*n* given in parentheses).

Serosal incubation medium	5-Hydroxytryptamine response	
	I_{sc}	Resistance
Normal Ringer	62.3 ± 2.9	58.9 ± 3.6 (37)
Normal Ringer + neuraminidase	59.1 ± 3.8	58.6 ± 4.4 (6)
Normal Ringer + AcNeu-aldolase	59.9 ± 4.2	60.1 ± 5.2 (5)
Ca^{2+}-free Ringer + 1 mM EDTA	56.9 ± 4.4	55.2 ± 3.4 (5)
Ca^{2+}-free Ringer + 1 mM EDTA + neuraminidase	16.3 ± 2.2	18.7 ± 2.8 (8)
Ca^{2+}-free Ringer + 1 mM EDTA + AcNeu-aldolase	56.2 ± 4.6	57.4 ± 5.1 (5)
Ca^{2+}-free Ringer + 1 mM EDTA + neuraminidase + AcNeu-aldolase	8.1 ± 2.1	7.6 ± 1.8 (8)

TABLE II

THE EFFECT OF NEURAMINIDASE ON THE RESPONSE OF FROG SKIN TO THE SEROSAL ADDITION OF 100 mU/ml OXYTOCIN, 10^{-6} M ADRENALIN AND 10^{-6} M ALDOSTERONE

The method is as described in Table I except that the above hormones were added to the serosal side instead of 10^{-5} M 5-hydroxytryptamine. Responses are calculated as the maximum % change (+ = increase; − = decrease) in I_{sc}/resistance ± S.E.M.

		Serosal incubation medium		
		Normal Ringer	Ca^{2+}-free Ringer + 1 mM EDTA	Ca^{2+}-free Ringer + 1 mM EDTA + neuraminidase
Oxytocin response	I_{sc}	+ 110.3 ± 6.1	+ 98.8 ± 7.2	+ 96.7 ± 5.1
	Resistance	− 8.1 ± 3.2	− 6.2 ± 2.8	− 7.3 ± 3.1
		(*n* = 5)	(*n* = 5)	(*n* = 5)
Adrenalin response	I_{sc}	+ 81.6 ± 6.8	+ 79.2 ± 3.9	+ 75.3 ± 5.2
	Resistance	+ 26.1 ± 4.3	+ 22.4 ± 4.1	+ 23.2 ± 5.0
		(*n* = 7)	(*n* = 7)	(*n* = 7)
Aldosterone response	I_{sc}	+ 40.1 ± 3.1	+ 36.1 ± 2.9	+ 37.8 ± 3.7
	Resistance	− 9.3 ± 1.8	− 10.6 ± 2.1	− 12.7 ± 1.6
		(*n* = 5)	(*n* = 5)	(*n* = 5)

lowing the above method but adding either 100 mU/ml oxytocin; 10^{-6} M adrenalin-HCl or 10^{-6} M aldosterone (all obtained from Sigma Co.) to the serosal medium instead of 5-hydroxytryptamine. Recovery of the 5-hydroxytryptamine responses in enzyme treated tissue was investigated by replacing the 5-hydroxytryptamine-containing serosal solution with either normal Ringer (control) or normal Ringer containing 10^{-4} M AcNeu, incubating for 2 h and then adding 10^{-5} M 5-hydroxytryptamine. Short circuit current and membrane potential were recorded manually and responses calculated as the maximum percentage change in I_{sc} and ohmic resistance across the tissue.

As indicated in Table I neuraminidase reduces both the active transport response and the chloride permeability response induced by 5-hydroxytryptamine in a parallel manner, however reduction of the responses occurs only in the absence of Ca^{2+} and presence of EDTA. This suggests that the receptor is closely associated with a component containing sialic acid and that Ca^{2+} are bound to it in such a manner as to stabilise it against enzyme attack; this is reasonable since Ca^{2+} are known to crosslink carbohydrate headgroups of glycolipids, glycoproteins and neighbouring membrane components via negatively charged carboxyl group residues on sialic acids leading to condensation and immobilisation of the headgroups within the membrane [5]. AcNeu-aldolase alone has no effect on the 5-hydroxytryptamine responses, however in combination with neuraminidase (and absence of Ca^{2+}) further decreases the 5-hydroxytryptamine responses suggesting that the receptor is associated with AcNeu residues and that in the presence of neuraminidase alone some resynthesis of receptor takes place utilising the released AcNeu, thus the 5-hydroxytryptamine responses are diminished but not abolished. In the presence of neuraminidase + AcNeu-aldolase the released AcNeu is degraded and hence no longer available for receptor synthesis leading to a further reduction in the 5-hydroxytryptamine responses; the responses do recover however when such tissue is incubated with AcNeu: addition of 5-hydroxytryptamine following a 2 hour incubation with 10^{-4} M AcNeu induces a 29.9% ± 3.1% increase in I_{sc} and a 26.3% ± 2.9% increase in transepithelial resistance compared with control recovery responses of 12.3% ± 2.1% (I_{sc}) and 9.2% ± 1.8% (resistance) indicating that receptor synthesis does take place in the tissue and that AcNeu greatly increases the rate of synthesis.

Neuraminidase treatment could have two effects in the tissue: first by removal of sialic acids (AcNeu) specifically degrade the 5-hydroxytryptamine receptor or secondly, since sialic acids, by virtue of their strong negatively charged carboxyl group, probably influence the configuration of the cell membrane by interactions in the glyco-calyx [5, 6], their removal would lead to alterations in membrane structure thus non-specifically changing hormone receptor sites. As Table II indicates the neuraminidase/AcNeu-aldolase effects are specific to the 5-hydroxytryptamine receptor since they have no effect on the response of the other hormones tested; thus it seems likely that neuraminidase specifically degrades the 5-hydroxytryptamine receptor by the removal of *N*-acetylneuraminic acid.

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