Table 1 Isoelectric focusing of developing rat brain choline acetyltransferase

Preparation of choline acetyltransferase	*Distribution of choline acetyltransferase activity* (%) pH 7.1–7.6	pH 7.6–8.0	pH 8.0–8.7
Adult brain (A)	14.38 ± 4.3	28.30 ± 6.7	52.85 ± 5.4
Adult brain (B)	10.53 ± 3.3	17.48 ± 3.6	64.14 ± 7.2
Immature brain (A)	31.18 ± 3.7	43.81 ± 5.9	18.97 ± 2.4
Immature brain (B)	25.20 ± 2.4	29.29 ± 6.2	19.33 ± 0.4

The enzyme preparations were either: (A) high speed supernatant of brain homogenate, or (B) partially purified as described by Malthe-Sørenssen & Fonnum (1972). Isoelectric focusing was carried out in 110 ml LKB columns (4°C), pH 6–9.5, 400 V for 46 hours. The distribution of ChA activity in the different pH ranges is expressed as a % of the total activity recovered after focusing. Residual activity was largely recovered in pH 6.5–7.1 range. Results are means ± s.e. mean of four focusing experiments in each case.

rat, may be related to the appearance of increased quantities of a basic isoenzyme of ChA. A molecular basis for the efficient coupling of sodium-dependent high affinity uptake and ChA will be discussed in light of these results.

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References

BARKER, L.A., DOWDALL, M.H. & MITTAG, T.W. (1975). Comparative studies on synaptosomes: high-affinity uptake and acetylation of N-(Me-^{3}H) choline and N-(Me-^{3}H)N-hydroxyethylpyrrolidinium. *Brain Res.*, **86**, 343–348.

BURGESS, E.J. & PRINCE, A.K. (1977). The effect of reserpine on choline acetylation and high affinity choline uptake in the rat brain. *Br. J. Pharmac.* In press.

FONNUM, F. (1975). A rapid radiochemical method for the determination of choline acetyltransferase. *J. Neurochem.*, **24**, 407–409.

MALTHE-SØRENSSEN, D. & FONNUM, F. (1972). Multiple forms of choline acetyltransferase in several species demonstrated by isoelectric focusing. *Biochem. J.*, **127**, 229–236.

POTTER, L.T. & MURPHY, W. (1967). Electrophoresis of acetylcholine, choline and related compounds. *Biochem. Pharmac.*, **16**, 1386–1388.

Activation of high affinity choline uptake in sympathetic ganglia by potassium depolarization

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There is evidence to suggest that high affinity uptake processes for choline are associated with cholinergic nerve terminals. Sympathetic ganglia accumulate choline by both high and low affinity uptake processes but we found that denervation of ganglia 10 to 14 days before their removal had no effect on the high affinity choline uptake process (Bowery & Neal, 1975). In the present study we have examined the effect of potassium depolarization on choline transport in ganglia and have found an association between cholinergic nerve terminals and a high affinity choline uptake process.

Isolated rat superior cervical ganglia were desheathed and given a preliminary incubation at 37°C for 30 min in Krebs bicarbonate Ringer. Then [^{3}H]-choline (1 μ Ci/ml) was added to give a final concentration of 0.1 μM or 100 μM and the incubations were continued for 10 minutes. Finally, the ganglia were dissolved in Soluene (Packard) and the radioactivity was measured by liquid scintillation counting. When ganglia were depolarized, potassium chloride (40 mM) was included in the medium both during the preliminary incubation and incubation period. The results are summarized in Table 1.

Potassium depolarization increased [^{3}H]-choline uptake by the high affinity uptake process but decreased transport by the low affinity process. Chronic denervation, absence of sodium or calcium ions in the medium, and increased magnesium ion concentration (20 mM), not only abolished the potassium induced activation of the high affinity choline uptake process, but changed the response to inhibition.

One explanation for these results is that potassium depolarization causes a calcium dependent release of

Table 1 Effect of potassium depolarization on [^{3}H]-choline uptake by rat sympathetic ganglia

Composition of medium for preliminary incubation and incubation	*Choline uptake* 'High affinity uptake' T/M	% control	'Low affinity uptake' T/M	% control
Control (Krebs bicarbonate Ringer)	6.9 ± 0.4	100	2.68 ± 0.14	100
KCl (40 mM)	9.9* ± 0.8	142*	2.12 ± 0.10*	73*
KCl, Ca free medium	5.1* ± 0.5	68*		
KCl + $MgSO_4$ (20 mM)	5.2* ± 0.2	64*		
KCl, Na free medium	3.5* ± 0.3	51*		
KCl, denervated ganglia	4.5* ± 0.3	54*		
Denervated ganglia (controls)	6.0 ± 0.3	112	2.70 ± 0.27	108

The high and low affinity uptake of choline was estimated by incubating ganglia in a low (0.1 μM) or high (100 μM) concentration of [^{3}H]-choline respectively. The results are expressed as both the tissue : medium ratio (T/M = dpm.g^{-1} wet weight/dpm.ml^{-1}) and as the percentage of uptake in control ganglia not exposed to KCl (% control). The 'T/M column' does not necessarily correspond exactly with the '% control column' since only the latter results are calculated from paired controls. The results (T/M) are the mean ± s.e. mean of 6 to 18 determinations.

* Significantly different from controls ($P < 0.0.5$ and > 0.001).

ACh from nerve terminals, which then results in activation of a high affinity, sodium dependent, uptake process for choline.

A.J. Higgins is an MRC student.

Reference

BOWERY, N.G. & NEAL, M.J. (1975). Failure of denervation to influence the high affinity uptake of choline by sympathetic ganglia. *Br. J. Pharmac.*, **55**, 278P.

Differentiation of neurogenic inhibition from ATP-responses in guinea-pig taenia caeci

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Twin 3–4 cm preparations were suspended in Krebs-Henseleit (1.5 mM Mg^{++}; 30°C, to reduce spontaneous tone-fluctuations) and contracted by carbachol, 27–164 nM. ATP-tachyphylactic muscles were discarded. ATP-relaxations persisted after phenoxybenzamine (2.3 μM) and pindolol (4–40 μM) but were reversibly antagonized by phentolamine, (2.6–13 μM) acting by an unknown mechanism not involving classical adrenoceptors of this muscle.

Neurogenic inhibition. To eliminate the participation of adrenergic fibres and to single out non-adrenergic inhibitory transmission, unatropinized preparations were rendered insensitive to the relaxing effect of phenylephrine, isoprenaline or noradrenaline by combined α-+β-adrenoceptor block following phenoxybenzamine-treatment (2.3 μM; 15 min) and the introduction of pindolol (40.3 μM). Tetrodotoxin-susceptible inhibitions induced by single 0.1–0.5 ms pulses were compared with relaxations induced by low ATP-concentrations (0.14–1.44 μM for 30 s). In all of 11 experiments, phentolamine (13 μM) greatly reduced ATP-relaxations but left neurogenic inhibitions usually unaltered (Figure 1), or potentiated or slightly reduced.

These results confirm in a different way that illustrated in a 'short report' by Rikimaru, Fukushi & Suzuki (1971), who used 10 times more phentolamine (135 μM); and atropine but no phenoxybenzamine or pindolol. Satchell, Burnstock & Dann (1973) (using guanethidine and hyoscine) failed to confirm this in 3 of 5 experiments with phentolamine (45 μM) and in order to achieve ATP-block raised phentolamine concentration to 180 μM, which then depressed transmission as well. But, under the present conditions, phentolamine in the much lower concentration of 13 μM has regularly differentiated neurogenic from ATP-responses. This, and the frequent occurrence of marked tachyphylaxis to ATP suggest that the non-adrenergic inhibitory transmission is not purinergic and may be akin to autonomic inhibitory transmission elsewhere, e.g. retractor penis, where ATP is excludable because it contracts whereas