neurotransmitter candidates on the PG synthesizing ability of synaptosomes.

Twenty per cent (w/v) homogenates of rat brain tissue (excluding cerebellum) were prepared in 0.32 M sucrose, and crude synaptosomes (P_2 fraction) prepared as described by Whittaker (1969). An aliquot (200 µl) of the synaptosome suspension (containing approx. 4 mg protein) was added to 1.3 ml Krebs bicarbonate medium (pH 7.4) containing ascorbic acid (1 mg/ml), isoniazid (10 µM) and, where appropriate, the substance under investigation (Table 1). Synaptosomes were incubated for 5 min at 37°C under 95% O_2 : 5% CO_2 followed by rapid centrifugation. To each supernatant was added marker amounts (2–5 pg) of [^{3}H]-PGE_2 or [^{3}H]-PGF_2 and the PG's released into the incubation media assayed, using a modification of the method of Hillier & Dilley (1974). Briefly, this involves selective extraction, column chromatography separation of PGE and PGF groups and radioimmunoassay using antibodies with relatively good selectivity for the PG's under investigation.

The results show that 100 µM amounts of NA, dopamine and adrenaline signficantly stimulate the generation of PGE by synaptosomal tissue. In another experiment NA also significantly stimulated PGF synthesis.

The effect is selective as acetylcholine, 5-HT and K^+ depolarization were without a stimulatory effect in this system.

We conclude that (a) contrary to the findings of Raffel *et al.* (1976) noradrenaline and dopamine do stimulate prostaglandin formation in rat brain synaptosomes, (b) this strengthens the link for PGEs exerting a positive feedback on NA release on central nerve terminals as described by Roberts & Hillier (1976).

We thank Dr J. Pike, The Upjohn Co. for the gift of prostaglandins.

References

HEDQVIST, P. (1976). Effects of prostaglandins on autonomic neurotransmission. In *Prostaglandins: Physiological, Pharmacological and Pathological Aspects*, ed. Karim, S.M.M., pp. 37–62. Lancaster: M.T.P.

HILLIER, K. & DILLEY, S.R. (1974). Separation and radioimmunoassay of $F_{2\alpha}$ prostaglandins using silica gel microcolumns. *Prostaglandins*, **5**, 137–150.

RAFFEL, G., CLARENBACH, P., PESKAR, A. & HERTTING, G. (1976). Synthesis and release of prostaglandins by rat brain synaptosomal fractions. *J. Neurochem.*, **26**, 493–498.

ROBERTS, P.J. & HILLIER, K. (1976). Facilitation of noradrenaline release from rat brain synaptosomes by prostaglandin E_2. *Brain Res.* (in press).

WHITTAKER, V.P. (1969). The subcellular fractionation of nervous tissue. In *The Structure and Function of Nervous Tissue*, ed. Bourne, G.H., Vol. 3, pp. 1–24. New York: Academic Press.

Ontogenesis of muscarinic receptor sites in rat brain

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Cholinergic neurotransmission in the central nervous system does not appear to be fully functional until the second week of life. Thus rats do not respond to injections of scopolamine with changes in locomotor activity until after the 15th day of life (Campbell, Lytle & Fibiger, 1969) and whilst scopolamine has a synergistic effect on amphetamine-induced gnawing in 30-day-old rats, this synergism could not be seen in 10-day-old rats (McGeer, Fibiger & Wickson, 1971). Both acetylcholinesterase and choline acetyltransferase (ChAc) are present in the rat neostriatum 4 days after birth although ChAc activity is very low (McGeer *et al.*, 1971; Guyenet, Beaujouan & Glowinski, 1975). However, it is possible that the lack of response to scopolamine indicates the absence of the muscarinic cholinoceptor on the postsynaptic neuron rather than the immaturity of the presynaptic pathway. This possibility has been examined by studying the binding of [^{3}H]-atropine to homogenates of neonatal rat cerebral cortex and caudate nucleus.

Homogenates of cerebral cortex and caudate nucleus in Krebs-Henseleit solution were preincubated for 15 min at 37°C before the addition of [^{3}H]-atropine (Radiochemical Centre, Amersham; 245 mCi mmol^{-1}). After a further 15 min the incubation was terminated by centrifugation at 14,000 *g* for 30 seconds. The surface of the pellet was rinsed twice with Krebs-Henseleit solution and radioactivity in the pellet was determined by liquid scintillation counting. Two series of samples were incubated together and one of these series contained 10 µM propylbenzilylcholine, a potent antimuscarinic compound, throughout the experiment. Receptor-specific binding of [^{3}H]-atropine was taken to be that fraction of binding which was abolished by the presence of the propylbenzilylcholine.

Receptor-specific binding of [^{3}H]-atropine was present in both the caudate nucleus and cerebral cortex of 5-day-old rats in concentrations which were 20–30% of the adult values. The concentrations increased with age and by 16 days had reached approximately 60% of the adult concentrations of receptor sites which were attained by the fifth week of life. Throughout, the caudate nucleus contained a higher concentration of receptor sites than the cerebral cortex.

These results indicate that the lack of response to scopolamine in young rats is not a consequence of the absence of the muscarinic cholinergic receptor though it is possible that the receptor is not functionally connected to the processes which are responsible for mediating the changes in neuronal activity produced by cholinergic agents.

I am indebted to Caroline Middleton for skilful technical assistance.

References

CAMPBELL, B.A., LYTLE, L.D. & FIBIGER, H.C. (1969). Ontogeny of adrenergic arousal and cholinergic inhibitory mechanisms in the rat. *Science*, **166**, 635–637.

GUYENET, P.G., BEAUJOUAN, J.C. & GLOWINSKI, J. (1975). Ontogenesis of neostriatal cholinergic neurones in the rat and development of their sensitivity to neuroleptic drugs. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **288**, 329–334.

McGEER, E.G., FIBIGER, H.C. & WICKSON, V. (1971). Differential development of caudate enzymes in the neonatal rat. *Brain Res.*, **32**, 433–440.

Analysis of end-plate current fluctuations produced by acetylcholine and acetylmonoethylcholine in rat muscle

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The characteristics of the ion channels that are opened by agonists in the frog muscle end-plate have been studied by analysis of the fluctuations about the mean end-plate voltage, or current, produced by the drugs (Katz & Miledi, 1972; Anderson & Stevens, 1973; Colquhoun, Dionne, Steinbach & Stevens, 1975). Similar experiments have now been conducted on rat diaphragm muscle fibres (Sakmann, 1976).

Strips of rat diaphragm were bathed in Krebs' solution containing tetrodotoxin (300 nM), at temperatures between 13° and 25°C. End-plate currents were recorded by a voltage clamp method. Analysis of current fluctuations was carried out on 10 s samples, filtered below 0.5 Hz and above 500 Hz. Digital samples were taken at 1 ms intervals. Agonists were applied by perfusion from a wide tipped micropipette (Cooke & Quastel, 1973) so as to produce end-plate currents of 10–90 nA.

The decay of spontaneous miniature end-plate currents (MEPC) showed no consistent deviation from a single exponential; and, equivalently, spectral density curves for both acetylcholine (ACh), and for acetylmonoethylcholine (AMECh) showed only slight deviation from a single Lorentzian form, so a single time constant could be inferred. The time constants for both MEPC decay (τ_{MEPC}) and for current fluctuations (τ_{ACh} or τ_{AMECh}) decreased with temperature ($Q_{10} = 3.26 \pm 0.63$), and increased as the membrane was hyperpolarized (109 ± 7 mV for an e-fold change in τ). These values are similar to those for frog muscle.

At 20°C, and −80 mV membrane potential, τ_{MEPC} was 1.6 ms. This appeared to be slightly (20–50%) slower than τ_{ACh}, though the size of the discrepancy depended on the method of curve fitting used for spectra. The simplest, but not the only, explanation for such a discrepancy would be that the acetylcholine concentration in the synaptic cleft did not fall rapidly compared with the lifetime of an open channel.

The ratio τ_{AMECh}/τ_{ACh} was 0.56 ± 0.05 (15); this can be interpreted as meaning that the average lifetime of an open ion channel, when the channel is opened by AMECh, is about 56% of its mean lifetime when opened by ACh. This ratio is similar to the ratio of decay time constants for normal (ACh) and 'false' (AMECh) MEPCs observed by Large & Rang (1976).

The mean conductance (γ) of a single open ion channel, may, under certain assumptions, be inferred from the variance of current fluctuations. No evidence was found for a difference in conductance between channels opened by ACh and AMECh; the values were $\gamma_{ACh} = 24.9 \pm 1.2$ pS ($n = 36$) (very similar to the value reported for frog muscle by Colquhoun *et al.*, 1975), and $\gamma_{AMECh} = 26.7 \pm 1.4$ pS ($n = 16$).

I am indebted to Dr Colin Mercer, Institute of Sound and Vibration Research, Southampton University, for data analysis facilities.