at high doses.

The rate of loss of actvity of prostaglandins in the skin was determined by measuring bradykinin-induced exudation in sites pre-injected with prostaglandins at various times. In spite of the difference in stability between PGI_2 and PGE_2 in aqueous solution, the times for half activity loss were similar: PGE_2 18.7 ± 3.0 ($n = 5$) min; PGI_2 12.0 ± 3.0 (n = 3).

These results suggest a possible role for PGI_2 in inflammation.

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A comparison of 3 methods used for measuring the overflow of noradrenaline in the mouse isolated stimulated vas deferens

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The following three methods for measuring the overflow of noradrenaline from the stimulated vas deferens have been used:

(1) Bioassay using the superfused rabbit abdominal aorta (Hughes, 1972).
(2) Radioenzymic method converting released noradrenaline to radioactive adrenaline with phenylethanolamine N-methyl transferase, using [^{3}H]-S-adenosyl methionine as methyl donor. (Henry, Starman, Johnson & Williams, 1975).
(3) Pre-loading with [7–^{3}H]-(-)-noradrenaline.

Measuring:
- (a) Total ^{3}H overflow
- (b) [^{3}H]-catechol overflow
- (c) [^{3}H]-noradrenaline and its metabolites overflowing

(Graefe, Stefano & Langer, 1973).

Methods 1 and 2 have the advantage that they measure only and all of the noradrenaline released from the tissue; whereas method 3(a) commonly used for measuring noradrenaline overflow actually measures labelled noradrenaline only, together with any of its metabolites. Basically therefore, methods 1 and 2 are superior to method 3, but with small tissues such as the mouse vas deferens it is difficult (impossible in our hands) to measure resting overflow.

Method 3 has the advantage of being the most sensitive, but is not applicable to patients whereas method 2 is and has been employed by Sever, Osikowska, Birch & Tunbridge (1977). Method 3(a) is not very informative and may be positively misleading because it measures a number of variables (i.e. radioactive noradrenaline and its metabolites). Thus, Stjärne (1975) and Marshall, Nasmyth & Shepperson (this meeting) using this method in the presence of uptake blockers to try to limit metabolism and to measure release as opposed to overflow, found that clonidine (2.8–11.2nM) did not reduce the overflow of tritium ([^{3}H]-noradrenaline implied in Stjärne's case). However when the tritiated metabolites were separated out as in method 3(c) the noradrenaline overflow was reduced by more than 50% while the overflow of metabolites was increased. Thus though method 3(c) measures only tritiated noradrenaline it does have the advantage that it also measures the tritiated metabolites and is superior to method 3(b) which does not separate the noradrenaline from other catechols.

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Presynaptic inhibition of acetylcholine release from cholinergic neurones in the myenteric plexus of the guinea-pig ileum

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The isolated ileum of the guinea-pig suspended in Krebs solution releases acetylcholine (ACh) spontaneously into the surrounding fluid. This ACh has its origin in the intramural nerve plexus (Johnson, 1963; Paton & Zar, 1968). Johnson (1963) found that when the ACh from such ileal segments was collected during 5, 10, 20, or 40 min periods the total amount recovered increased with the increasing collection time, but the rate of spontaneous release fell progressively.

The possibility arises that the accumulation of ACh in the synaptic cleft inhibits the further output of the transmitter and in support of this suggestion Kilbinger & Wagner (1975) showed that oxotremorine caused an atropine sensitive reduction in spontaneous output of ACh from guinea-pig ileum treated with physostigmine or DFP. Kilbinger & Wagner also postulated that oxotremorine acted on a neuronal receptor responsible for feedback control of ACh release from the ileum at rest.

A different observation, this time on the electrically transmurally stimulated guinea-pig ileum, seems relevant. When the ileum was contracted submaximally by a train of stimuli (0.2 Hz) until constant responses were obtained, and then with the current off a dose of ACh was added for 30 s and then washed out, electrical restimulation at this point resulted in smaller twitches than those obtained before the ACh. Recovery of the twitch responses after ACh to control height took 8–10 minutes. ACh caused a dose-dependent inhibition of the twitch response to electrical stimulation over the concentration range 0.85 nM-68.4 μM.

The increase in efflux of [^{3}H]-ACh on electrical field stimulation of ileal strips pretreated with 99 μM [^{3}H]-choline for 2 h, was inhibited by exogenous ACh (1.7 μM) in Krebs containing physostigmine (0.18 μM) and hemicholinium (17 μM). This inhibition of [^{3}H]-ACh efflux was reversed by atropine (0.35 μM).

These experiments appear to provide direct evidence that exogenous ACh can inhibit the output of transmitter ACh by an action on a presynaptic muscarinic cholinoceptive receptor.

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