The responses to glutamate and aspartate were very similar—a rapidly plateauing depolarization with little or no change in G_M with moderate doses. NMDA gave a 'triangular' shaped response accompanied by a very large decrease in G_M (Figure 1), even larger than that seen with D-homocysteate (Lambert, Flatman & Engberg, 1978). Long applications of NMDA evoked persistent firing.

Kainate was extremely potent—ejecting currents of 5-20 nA routinely evoked depolarizations of 30–40 mV. During the slow climbing phase of the response there was little change in G_M (an increase in G_M when allowing for anomalous rectification). The response would continue to climb through a short phase of firing and finally arrive at a plateau—usually at a membrane potential of −20–−30 mV. At the plateau G_M was immeasurably large. Recovery was slow and seldom complete (except with *very* small doses). Motoneurone axons were essentially unresponsive to kainate.

Responses to glutamate and aspartate are qualitatively very similar, while that to NMDA is so dissimilar to both as to question that aspartate and NMDA act on the same receptor (even allowing for the absence of NMDA uptake). Moreover, the phenomenal potency, slow onset of action, large G_M increase and irreversibility characteristic of kainate depolarizations poses the questions whether kainate interacts with a 'glutamate' or an 'aspartate' receptor.

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Do primary afferent terminals have acidic amino acid receptors?

R.H. EVANS & J.C. WATKINS

Department of Pharmacology, The Medical School, University of Bristol, Bristol BS8 1TD

Recordings from dorsal roots of rat isolated spinal cords suggest that afferent terminals are depolarized not only by GABA and other neutral amino acids but also by a range of excitatory amino acids (Evans 1978). However it is possible that the dorsal root depolarizations produced by acidic amino acids are not generated directly via presynaptic receptors for these substances but indirectly via release of some depolarizing substance such as potassium or a transmitter into extracellular space. Provided the rate of reuptake or synthesis of such a substance was slower than the release, then such indirect responses would be expected to fade during prolonged superfusion of excitant (as the released substance is flushed away) and subsequent applications of excitant should be reduced or abolished.

Prolonged treatment (30–120 min) of rat isolated hemicords with the potent excitant N-methyl-D-aspartate (NMDA, 25–200 μM), had an effect consistent with the above possibility. Such applications of NMDA produced depolarization recorded in dorsal roots which faded back to the resting polarity with a half time of 5–10 minutes. However, the concomitant depolarization recorded from ventral roots showed only a partial fade, with a time course similar to that shown by dorsal roots, and ventral roots remained depolarized at equilibrium. This residual ventral root depolarization was maintained for several hours, suggesting that motoneurones are depolarized directly by NMDA.

Similar effects were observed with hemisected frog spinal cords, and in both species, after prolonged NMDA treatment, no depolarizing responses could be evoked by NMDA at a thousand times the usual threshold level (1 μM). In contrast, kainate still depolarized dorsal (but not ventral) root fibres of frog or rat isolated hemicords in the continued presence of NMDA, although NMDA treated tissues were at least 10 times less sensitive to kainate than controls.

Dorsal root sensitivity to GABA was not significantly altered by NMDA treatment, and in frog preparations during such treatment GABA responses were similar to those of dorsal roots attached to control hemicords rather than to those of isolated dorsal roots, which had a much longer time course. This indicates that afferent terminals are unlikely to have been damaged by the NMDA treatment.

These effects suggest either that presynaptic terminals desensitize to NMDA and only partially to kainate or that these terminals have kainate receptors but not NMDA receptors. If the latter possibility is correct, it would indicate that depolarizations of affer-

ent terminals evoked by NMDA and similar excitants are caused indirectly via the release of another substance, possibly potassium into extracellular space.

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Clinically-used dyes are inhibitors of prostaglandin E_2 inactivation in rat isolated lung

Y.S. BAKHLE

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

Indicator dyes such as bromcresol green and thymol blue prevent the inactivation of prostaglandin $F_{2\alpha}$ (Bito & Baroody, 1974) and prostaglandin E_2 (Bakhle, Jancar & Whittle, 1978) in rat isolated lungs. Other dye molecules are used in clinical practice for a variety of purposes and we have therefore studied the effect of some clinically used dye molecules on the pulmonary inactivation of prostaglandin E_2.

The inactivation of prostaglandin E_2 on a single passage through rat isolated lungs perfused with Krebs solution (8 ml/min) was measured by bioassay on the hamster stomach strip (Bakhle *et al.*, 1978). In each experiment the inactivation of prostaglandin E_2 was measured before, and 20 min after infusing the dye under investigation through the pulmonary circulation. The effect, if any, of the dye was rapidly attained (within 15 min) and remained constant for as long as the dye was infused. After the dye infusion was stopped, the inactivation tended to return towards the original level but recovery from dye treatment was not systematically studied. The results summarised in the Table show that all the dyes inhibited prostaglandin E_2 inactivation, the least potent being methylene blue.

The effect of the dyes can also be demonstrated by injecting the dye during an infusion of prostaglandin E_2. For instance, a constant infusion of prostaglandin E_2 (2 or 20 ng/ml) through the pulmonary circulation produced no contraction of the assay tissue superfused with lung effluent. However, an injection of sulphobromophthalein (0.25 μmole; 210 μg) through the lung caused a contraction equivalent to about 10 or 100 ng prostaglandin E_2 respectively. Injection of this dose of sulphobromophthalein directly to the tissue was without effect.

The results show that these dyes, and perhaps others in clinical use, interfere with at least one physiological process and suggest that the assumption that they are pharmacologically inert may no longer be valid.

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Table 1 Inhibition of pulmonary inactivation of prostaglandin E_2 by dyes

Concentration required (μM) to produce 20% inhibition				
Indocyanine green	*Sulphobromophthalein*	*Phenol red*	*Evans blue*	*Methylene blue*
(775)	(838)	(354)	(961)	(356)
<0.4	2.5	9	7	90

These values have been taken from dose–effect studies using at least two doses of dye and at least 8 separate experiments. The number in brackets is the MW of the dye.