

C. J. EAVES

Medical Biophysics Unit,  
British Columbia Cancer Research Center  
and  
Department of Medical Genetics,  
University of British Columbia, Canada

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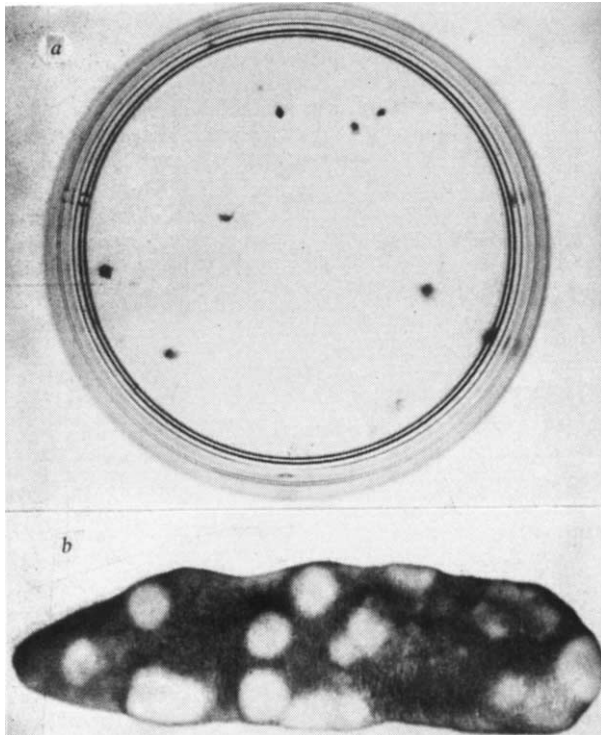


Fig. 2 a, Gross appearance of macroscopic bursts from 2-week flask cultured marrow seen in assay dishes after 12 d incubation (2 $\times$ ). b, Gross appearance of spleen (fixed in Tellyesniczky's solution) showing colonies produced 9 d after injection of eight macroscopic bursts (5 $\times$ ).

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## Evidence that substance P does not mediate slow synaptic excitation within the myenteric plexus

ELECTRICAL stimulation of presynaptic fibres to the so-called AH<sup>1</sup> or type II<sup>2</sup> myenteric neurones in guinea pig small intestine evokes a slow excitatory postsynaptic potential (e.p.s.p.) characterised by long-lasting depolarisation associated with increased membrane resistance and augmented excitability<sup>3</sup>. Two substances have been implicated as possible neurotransmitters for the slow e.p.s.p. Katayama and North reported that application of substance P to myenteric neurones mimicked the slow e.p.s.p.<sup>4</sup>, and J.D.W. and C.J.M. presented several lines of evidence for serotonin as the transmitter substance<sup>5,6</sup>. We now report that methysergide, a drug which abolishes both the slow e.p.s.p. and the action of exogenous serotonin<sup>5,6</sup>, does not affect the action of substance P on guinea pig myenteric neurones. The results suggest that substance P is unlikely to be the neurotransmitter which mediates the slow e.p.s.p.

We used conventional methods, which are described in detail elsewhere, to record intracellular electrical activity and to evoke the slow e.p.s.p. in myenteric ganglion cells of guinea pig small intestine<sup>3</sup>. Substance P from three different sources (Beckman, Serva and Sigma) and methysergide (Sandoz) were applied to the neurones by adding the drugs to the superfusion solution.

We tested substance P 41 times on 35 ganglion cells from 15 guinea pigs. The compound produced a dose-dependent membrane depolarisation in 30 of the ganglion cells and had no effect on 5 of the cells (Figs 1, 2). In 22 neurones, the depolarisation was accompanied by an increase in membrane resistance, with a decrease in 8. The increase in input resistance did not seem to be related to membrane rectification, and probably reflects decreased membrane conductance for potassium, as suggested by Katayama and North<sup>4</sup>. The membrane depolarisation reached a plateau, and after 45-60 s in substance P, repolarisation, apparently reflecting tachyphylaxis, began. The effects of substance P were reversed when the preparations were washed with drug-free Krebs solution (Fig. 1).

Substance P had the same dose-dependent effects in the presence and absence of methysergide (Fig. 2). On all occasions, the membrane depolarisation produced by the three concentrations of substance P in the presence of methysergide was not significantly different ( $P > 0.10$ ) from the values obtained in its

repopulating and differentiative potentialities of cells derived from macroscopic burst progenitors.

The broad distribution of CFU-S numbers in individual bursts is of interest, as it approaches that observed for CFU-S self-renewal in spleen colonies<sup>10,12</sup>. The number of cells plated in burst assay cultures was low. It thus seems most likely that the distribution observed was due to statistical fluctuations<sup>13</sup> rather than micro-environmental influences<sup>14</sup>. This does not negate the possibility that in complex cell systems, stem cell growth and differentiation can be locally influenced by other cell types in the immediate vicinity. It does, however, suggest that their effects can be studied using soluble factors. The high frequency of CFU-S per burst demonstrated here is an order of magnitude higher than that recently reported for mixed haematopoietic colonies of early fetal liver cell origin<sup>15</sup>. Thus, experiments to analyse the progeny of individual adult marrow stem cells stimulated *in vitro* are now possible. An immediate application will be to study the possible influence of various molecular factors on the self-renewal and differentiative behaviour of these stem cells.

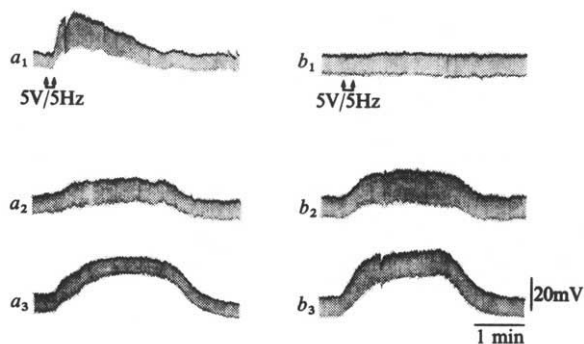
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R. K. HUMPHRIES  
P. B. JACKY  
F. J. DILL

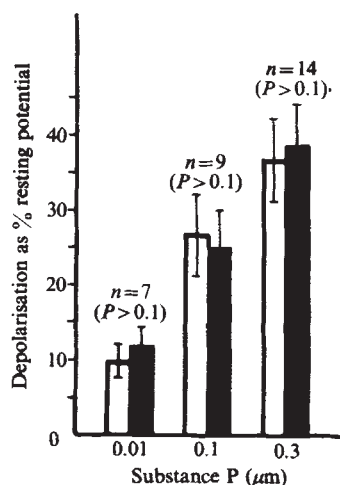
Department of Medical Genetics,  
University of British Columbia

Department of Medicine,  
University of British Columbia

A. C. EAVES



**Fig. 1** Effects of methysergide on the slow e.p.s.p. and on the action of substance P in a myenteric neurone of guinea pig small intestine. Each neurone was impaled with the microelectrode and the following procedure carried out. (1) Constant current, hyperpolarising pulses were continuously injected into the neurone at 1-s intervals to monitor changes in the neurone's input resistance. (2) Three concentrations of substance P (10, 100 and 300 nmol l<sup>-1</sup>) were applied to the neurone in succession, but in variable order. Each concentration of substance P remained in contact with the neurone for 2 min and was then washed from the superfusion system for a period of 5 min with drug-free Krebs solution before addition of the next concentration of substance P. (3) The slow e.p.s.p. was evoked by electrical stimulation of one of the interganglionic fibre tracts that entered the ganglion. (4) Methysergide 20 μmol l<sup>-1</sup> was then added to the superfusion solution. (5) After 5 min in the presence of methysergide, electrical stimulation was again applied to the fibre tract. The same stimulus parameters were used and the stimulating electrode remained in the same position on the fibre tract throughout the experiment on each neurone. (6) The three concentrations of substance P were then applied in the same manner in the continuous presence of methysergide. (7) The methysergide was washed from the superfusion system and electrical stimulation was again applied to the fibre tract. (8) Current-voltage relationship and rectifying properties of the neuronal membrane were examined. The microelectrode was sometimes dislodged from the cell before completion of the above sequence and this is reflected in the numerical data of Fig. 2. We also sometimes repeated the entire sequence on the same cell. *a*<sub>1</sub>, Slow e.p.s.p. evoked by application of a short train of stimulus pulses (arrows) to one of the fibre tracts that entered the ganglion. Increased amplitude of the electrotonic potentials produced by current injection (increase in baseline width) reflected an increase in the input resistance of the neurone during the depolarising phase of the e.p.s.p. One spike with a long-lasting hyperpolarising after-potential occurred at the peak of the e.p.s.p. *b*<sub>1</sub>, Fibre tract stimulation did not evoke a slow e.p.s.p. in the presence of methysergide. *a*<sub>2</sub> (100 nmol l<sup>-1</sup>), *a*<sub>3</sub> (300 nmol l<sup>-1</sup>). Dose-dependent depolarising action of substance P. *b*<sub>2</sub> (100 nmol l<sup>-1</sup>), *b*<sub>3</sub> (300 nmol l<sup>-1</sup>). The presence of methysergide did not reduce the dose-dependent action of substance P. This neurone in each case was exposed to substance P for 2 min and then substance P washed from the superfusion system.



**Fig. 2** Dose-dependent effect of substance P on the electrical potential across the membrane of guinea pig myenteric neurones in the presence and absence of 20 μmol l<sup>-1</sup> methysergide. The maximal amount of depolarisation produced by each concentration of substance P is expressed as the per cent of the resting potential before application of the peptide. Results given are mean values, s.e.ms, number of trials per concentration and the level of statistical significance. The arc sine transformation and Student's *t* statistics<sup>8</sup> were used to determine the level of significant difference between the means for each concentration of substance P in the presence and absence of methysergide. □, Substance P; ■, Substance P + methysergide.

absence. Similarly, the changes in input resistance produced by substance P were unaffected by methysergide. On the other hand, on the same cells, the presence of methysergide abolished the stimulus-evoked slow e.p.s.p. (Fig. 1), but this effect was reversed by washing the preparation with drug-free solution. Previous studies indicated that this blocking action of methysergide was due to a specific action at serotonin receptors and not a local anaesthetic action, because electrical stimulation still elicited spike discharge in the neurone when the slow e.p.s.p. was blocked in the presence of methysergide<sup>5,6</sup>.

Our observation that methysergide abolishes the slow e.p.s.p. without affecting the action of substance P makes it unlikely that substance P is the transmitter. Methysergide does block the action of serotonin and this, as well as several other lines of evidence, implicate serotonin as the neurotransmitter<sup>5</sup>. In the brain, the observation that substance P and serotonin occur within the same synaptic vesicles<sup>7</sup> suggests a functional relationship between the two substances; however, such a relationship has not yet been demonstrated.

P. GRAFE  
C. J. MAYER

Physiologisches Institut  
der Universität München,  
Pettenkoferstr. 12,  
D-8000 München 2, FRG

J. D. WOOD

Department of Physiology,  
University of Kansas Medical Center,  
Kansas City, Kansas 66103

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## Serum triggers a sequence of rapid ionic conductance changes in quiescent neuroblastoma cells

SERUM is required for the growth of nearly all animal cells in culture, but the mechanisms by which serum interacts with cells are largely unknown<sup>1,2</sup>. Evidence exists, however, that the primary site of action of the serum constituents is at the plasma membrane. For example, the first detectable events following serum stimulation of resting fibroblasts involve alterations in membrane transport, such as a stimulation of the (Na<sup>+</sup> + K<sup>+</sup>)ATPase<sup>3,4</sup> and an increase in the uptake of various nutrients<sup>5</sup>. Most of this evidence has been obtained using tracer flux techniques, but the relatively poor time resolution of this method (of the order of minutes) has precluded detection of dynamic membrane changes that may occur within seconds of serum addition. We have applied intracellular electrophysiological techniques in a search for rapid ionic membrane events following serum stimulation of mouse neuroblastoma cells. These cells stop growing (become 'quiescent') after serum removal and begin to extend neurites, but on re-addition of serum the neurites retract and cell division resumes<sup>6,7</sup>. Here we report that the immediate consequence of adding fetal calf serum (FCS) to quiescent neuroblastoma cells is a triphasic