A new example of a morphine-sensitive neuro-effector junction: adrenergic transmission in the mouse vas deferens

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Commentary by

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This paper showed for the first time that morphine acting through its specific mu-opiate receptor could inhibit both mechanical activity and noradrenaline output induced by transmural sympathetic nerve stimulation of the mouse isolated vas deferens. The Aberdeen team utilised this discovery in the search for the endogenous ligand of the opiate receptor resulting in the isolation of the enkephalins. This preparation is still widely used as a bioassay for opioids and other chemical messengers.

I joined the faculty of the University of Aberdeen in 1969 as a lecturer in the Department of Materia Medica and Experimental Medicine. Hans Kosterlitz had been made Professor of Pharmacology at the age of 65 with a remit to establish a new department of Pharmacology which I joined with Dr. Gordon Lees as founding faculty members. Graeme Henderson joined the embryo department as a Ph.D. student in 1970 to work on the possible relationship between opiate inhibition of neurotransmission and the properties of synaptic release at different neuro-effector junctions. The work of Kosterlitz's group on cholinergic transmission and my own on adrenergic transmission had converged with the findings that the output of transmitter per stimulus with stimulation frequency varied at different types of autonomic cholinergic or adrenergic junctions. Thus in the morphine sensitive, guinea-pig longitudinal muscle - myenteric plexus preparation the output per pulse of acetylcholine was greatest at single pulses and low stimulus frequencies whereas the corresponding (non-morphine sensitive) rabbit preparation showed little frequency dependence. I had shown that noradrenaline release per pulse increased with frequency at a number of adrenergic junctions which were morphine insensitive. It was Graeme's task to measure noradrenaline release from the cat nictitating membrane, the nerve-induced contractions of which had previously been shown by Trendelenburg (1957) and Cairnie, Kosterlitz & Taylor (1961) to be sensitive to morphine inhibition. Assisted by the invaluable advice and help of Professor John Thompson we showed for the first time that morphine does actually inhibit noradrenaline release after sympathetic stimulation and that the stimulus frequency output curve for this junction differed from others we had examined in that the output of noradrenaline remained constant over a wide frequency range.

The isolated nictitating membrane was an extremely difficult preparation requiring at least an hour of careful dissection and was clearly impracticable for detailed studies of the opiate receptor pharmacology. It was with some interest then, that I read a paper by Farnebo & Malfors (1971) which described the output of (^{3}H)-noradrenaline from the mouse vas deferens as being constant with the frequency of stimulation, just as we had found with the cat nictitating membrane. I suggested to Graeme that it might be worth testing the mouse vas for morphine sensitivity and I still remember the tremendous excitement engendered by the positive result of the first experiment. Hans Kosterlitz was delighted and we immediately pooled resources to exploit this new bioassay. Together with another of my students Frances Leslie (Hughes, Kosterlitz & Leslie 1975) we developed the preparation so that it could be used as a fully-quantitative bioassay allowing us to measure equilibrium dissociation constants for opiate antagonists and establish an excellent correlation between the rank orders of opiate agonist action in the mouse vas, guinea-pig ileum and brain (esti-

mated by antinociceptive activity). The relevance of the peripheral opiate receptor was beyond question in our eyes, although not accepted by everyone in the field, and allowed us to address the wider issues of the nature of opiate agonism and its relevance to endogenous mechanisms.

We were not alone in debating the relevance of the opiate receptor but I believe that we were the first to put the essential elements of opiate agonism, receptor stereospecificity and neuroeffector selectivity in a coherent framework leading to the successful testing of the hypothesis that there must be an endogenous opiate ligand for such a highly organised neurochemical system. The mouse vas deferens played a key role in our search for this ligand resulting in the isolation and identification of (met^5)- & (leu^5)-enkephalin (Hughes 1975; Hughes *et al.*, 1975). Other groups and most notably that of Lars Terenius at Uppsala University, Sweden had considered the possibility of an endogenous opiate ligand. However the use of opiate receptor binding techniques as used first by Terenius and later by Solomon Snyder's group at Johns Hopkins University was fraught with technical problems not the least being that unlike the bioassay it was not possible to have effective controls for non-specificity, a problem easily circumvented in the bioassay by using stereospecific opiate receptor antagonists.

The discovery of a new opiate receptor, the delta-receptor named for the mouse vas deferens, also followed on from observations first made with this preparation (Lord *et al.*, 1977). Again this discovery highlighted the power of the bioassay, whilst it is possible to define receptors according to their binding properties only a bioassay can lend a physiological significance to such a classification and this holds true today even with the new technology of expression cloning of receptors. The concept of what defines a receptor class has of course become immensely more complicated with our ability to identify closely-related cDNA sequences; is the orphanin receptor (ORL_1) an opioid receptor? It has a close homology (>50% identical cDNA sequence) with the mu-, delta- and kappa-opioid receptors and exhibits similar biochemical and pharmacological responses *in vitro*, including inhibition of neurogenic contractions of the mouse vas deferens, when activated by its endogenous ligand orphanin-FQ/nociceptin (Reinscheid *et al.*, 1995; Meunier *et al.*, 1995). However orphanin's actions cannot be blocked by naloxone which would have previously excluded it from the opioid family had it been identified by classical pharmacological techniques. The challenge for today's pharmacologists is to integrate modern molecular biological technology and information into the more classical aspects of the subject, both are equally important in establishing pharmacological relevance and utility.

As far as I know the significance of the frequency- transmitter output characteristics of morphine sensitive junctions was never satisfactorily explained (could it be due to endogenous enkephalin release?) but this abstruse scientific problem lead us into issues of much wider scientific significance which were solved using quantitative pharmacological tools and in particular the mouse vas deferens. Indeed the tradition continues at Aberdeen where Roger Pertwee and Angela Waterfield use the mouse vas deferens to study cannabinoid receptor pharmacology.

Hans Kosterliz died at the age of 93 on the 26th October 1996 after an extraordinarily fruitful career, the most active period coinciding with our collaboration when he was in his sixties and seventies. Graeme Henderson, who is now Professor of Pharmacology at Bristol, and myself will always be grateful to Hans for sharing with his younger colleagues not only his excitement for the opiate field but also his general zest for life which naturally involved much heated discussion and fine food and drink. Hans exemplified the highest standards of scientific scholarship and inquiry.

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