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SPECIAL REPORT

Ketamine potentiates 5-HT_3 receptor-mediated currents in rabbit nodose ganglion neurones

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The interaction of ketamine with the 5-HT_3 receptor of rabbit nodose ganglion neurones is described. Ketamine (3–30 μM) enhanced 5-HT_3 receptor-mediated currents recorded under voltage-clamp conditions. This action did not appear to be related to the known effect of ketamine of inhibiting 5-HT uptake.

Keywords: 5-HT_3 receptors; ketamine; rabbit nodose ganglion neurones

Introduction In some neurones of the peripheral and central nervous system, 5-HT_3 receptor activation elicits a membrane depolarization mediated by the opening of cation selective ion channels (Peters *et al.*, 1991). The dissociative anaesthetic, ketamine, voltage-dependently blocks both nicotinic (Maleque *et al.*, 1981) and N-methyl-D-aspartate (NMDA) -activated cation conducting channels (MacDonald *et al.*, 1987). Here, the actions of ketamine upon 5-HT_3 receptor-mediated currents recorded from isolated nodose ganglion neurones of the rabbit were examined. We now report that in contrast to its action on nicotinic and NMDA-activated ion channels, ketamine produces a dose-dependent potentiation of the 5-HT_3 receptor-mediated current.

Methods Adult New Zealand rabbits (3.5–6.0 kg) were anaesthetized with halothane, killed by exsanguination, and the nodose ganglia excised. Ganglia were enzymatically dissociated into a single cell suspension by the method of Ikeda *et al.* (1986), with minor modifications. The cells were maintained for 1–7 days in a culture medium comprising: Dulbecco's Modified Eagle's Medium (90% vol/vol) and heat inactivated foetal calf serum (10% vol/vol), supplemented with penicillin (5×10^4 iu l^{-1}), streptomycin (50 mg l^{-1}) and nerve growth factor (50 ng ml^{-1}). Agonist-evoked membrane currents were recorded at room temperature (17–22°C) under voltage-clamp conditions by use of a patch pipette in conjunction with either a List Electronics L/M EPC-7 converter headstage and amplifier or an Axoclamp 2A amplifier in discontinuous single electrode voltage-clamp mode (switching frequency 8–13 kHz). Cells were continuously superfused (2–4 ml min^{-1}) with a recording medium containing (in mM): NaCl 140, KCl 2.8, $CaCl_2$ 1.0, $MgCl_2$ 2.0 and HEPES 10 (pH 7.2). In some experiments LiCl totally replaced NaCl in the extracellular medium. The patch pipette solution comprised (in mM): CsCl (or KCl) 140, $MgCl_2$ 2.0, $CaCl_2$ 0.1, EGTA, 1.1 and HEPES 10 (pH 7.2). Agonists were applied either by pressure ejection (1.4×10^5 Pa, 5–20 ms duration, 0.1 Hz) from a second patch pipette, or by inclusion within the superfusate. All other compounds were applied by the latter route. Quantitative results are expressed as the arithmetic mean ± the standard error of the mean. The drugs used were obtained from the following sources: DL-2-aminophosphonovaleric acid (APV), 5-hydroxytryptamine (5-HT) creatinine sulphate complex (Sigma); 2-methyl-5-HT, ondansetron (Glaxo); ICS 205-930 ((3α-tropanyl)-1H-indole-3-carboxylic acid ester), methysergide (Sandoz); 1-phenylbiguanide (RBI); MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (Merck Sharp and Dohme); ketanserin (Janssen), ketamine (Parke-Davis), citalopram (Lundbeck) and paroxetine (SmithKline Beecham).

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Results In neurones voltage-clamped at a holding potential of −60 mV, 5-HT (10 μM) applied by pressure ejection evoked a transient inward current response associated with an increase in membrane conductance. Such currents were little affected by either bath applied methysergide (1 μM; $n = 6$) or ketanserin (1 μM; $n = 7$) but were reduced to 3.1 ± 0.8% ($n = 3$) and 0.3 ± 0.3% ($n = 6$) of control by ICS 205-930 (1 nM) and ondansetron (1 nM) respectively. Bath-applied ketamine reversibly and concentration-dependently potentiated the amplitude of submaximal inward currents evoked by pressure applied 5-HT (Figure 1a,b). Over the concentration range 3–30 μM, potentiation was well maintained throughout the period of ketamine application, but higher concentrations (100–300 μM) of the drug produced a complex effect consisting of an initial potentiation which was succeeded by a fall in current amplitude toward, or below, control values. Upon washout of high concentrations of ketamine (300 μM), a second phase of potentiation was often observed (Figure 1a). Potentiation by ketamine (30 μM) occurred at all holding potentials examined (−80 to +40 mV) and the drug had no effect upon the reversal potential (approx. −3.0 mV) of the 5-HT-induced response (Figure 1c,d). In neurones where ketamine (10–30 μM) produced a clear potentiation of 5-HT, APV (30 μM) had no effect ($n = 3$) whereas MK-801 (30 μM) slightly reduced the response to 93.3 ± 3.2% ($n = 3$) of control.

In cells sequentially challenged with rapidly superfused 5-HT (1 μM), 2-methyl-5-HT (3 μM) and 1-phenylbiguanide (30 μM), ketamine (10 μM) potentiated the amplitude of the inward current response evoked by each agonist (Figure 2a). Citalopram (0.01–1 μM) had no effect upon responses to pressure applied 5-HT ($n = 3$; Figure 2b). Neither the addition of citalopram (1 μM) or paroxetine (30 nM) to the bathing medium, nor the total replacement of extracellular Na^+ by Li^+, prevented the potentiating action of ketamine (10–30 μM; Figure 2b,c).

Discussion The dissociative anaesthetic, ketamine, blocks the cation-conducting channels activated by nicotine and NMDA (Maleque *et al.*, 1981; MacDonald *et al.*, 1987). In contrast, ketamine (3–30 μM) enhanced the 5-HT_3 receptor-mediated current of rabbit nodose ganglion neurones. Qualitatively, ketamine (30 μM) produced a similar effect in mouse nodose ganglion neurones, although the magnitude of the potentiation was much less than that of the rabbit and was often succeeded by an antagonism of the response (Malone, preliminary observations). Species differences in the properties of 5-HT_3 receptors (see Peters *et al.*, 1991) may account for this discrepancy. The effect of ketamine in the rabbit was not restricted to 5-HT, as currents evoked by the 5-HT_3 receptor agonists, 2-methyl-5-HT and 1-phenylbiguanide, were similarly potentiated. The ketamine-induced potentiation was not mimicked by the competitive NMDA receptor antagonist, APV, or by the NMDA ion channel blocker, MK-801.

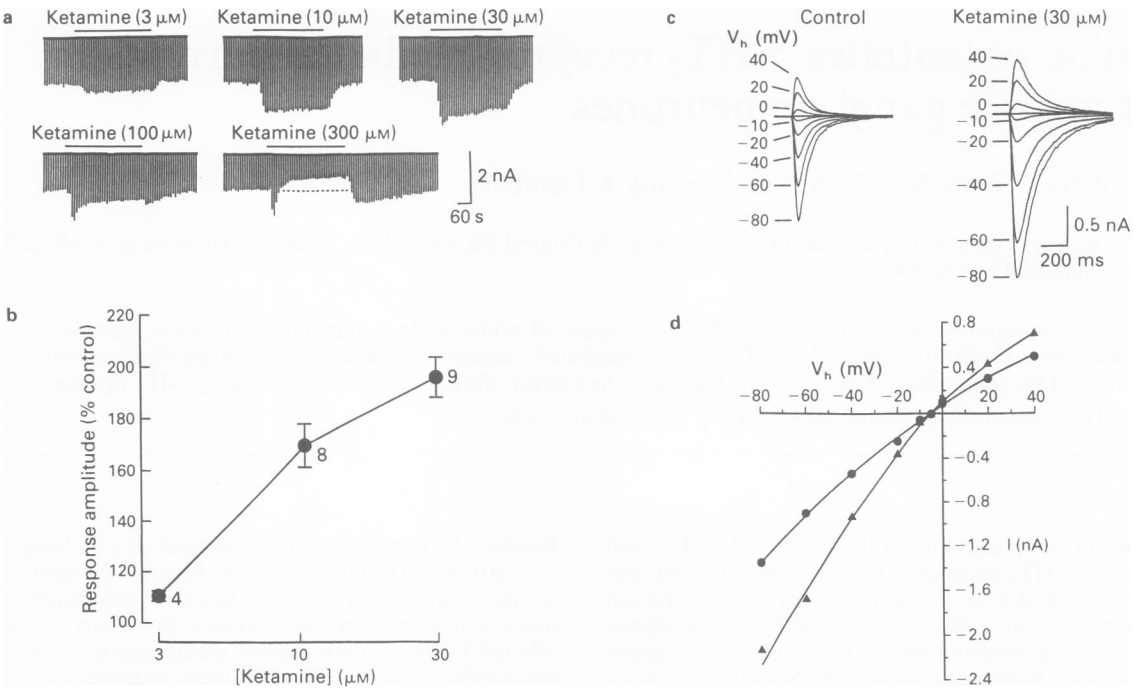

Figure 1 Ketamine potentiates 5-HT_3 receptor-mediated membrane currents in rabbit nodose ganglion neurones. (a) Traces illustrating the potentiation of responses to pressure applied 5-hydroxytryptamine (5-HT, 10 μM, 1.4×10^5 Pa, 0.1 Hz, Pa, 0.1 Hz, 10 ms duration) by bath-applied ketamine. Note that potentiation in response to high concentrations of ketamine (100–300 μM) is not maintained throughout the period of superfusion and is succeeded by blockade of the response in the case of 300 μM ketamine. A second phase of potentiation occurs upon washout of 300 μM ketamine. Currents were recorded at a holding potential of −60 mV and low pass filtered at 0.5 kHz. (b) Graph illustrating the concentration-dependence of the ketamine induced potentiation of currents evoked by 5-HT. Response amplitude, as a percentage of control, is plotted against the logarithm of ketamine concentration in the medium on a logarithmic scale. Each data point represents the mean of 4–9 observations performed on separate neurones. Vertical lines represent s.e.mean. (c) Traces illustrating the transmembrane current in response to pressure applied 5-HT, recorded at various holding potentials (V_h), and the influence of ketamine (30 μM) on such responses. Note that the leakage currents have been subtracted. (d) Graph representing the data from (c) shows the relationship between the amplitude of the 5-HT-induced current and holding potential under control conditions (●) and in the presence of 30 μM ketamine (▲). Note that the potentiation by ketamine occurs at all holding potentials, without influencing the $I_{5\text{-HT}}$ reversal potential (approx. −3 mV).

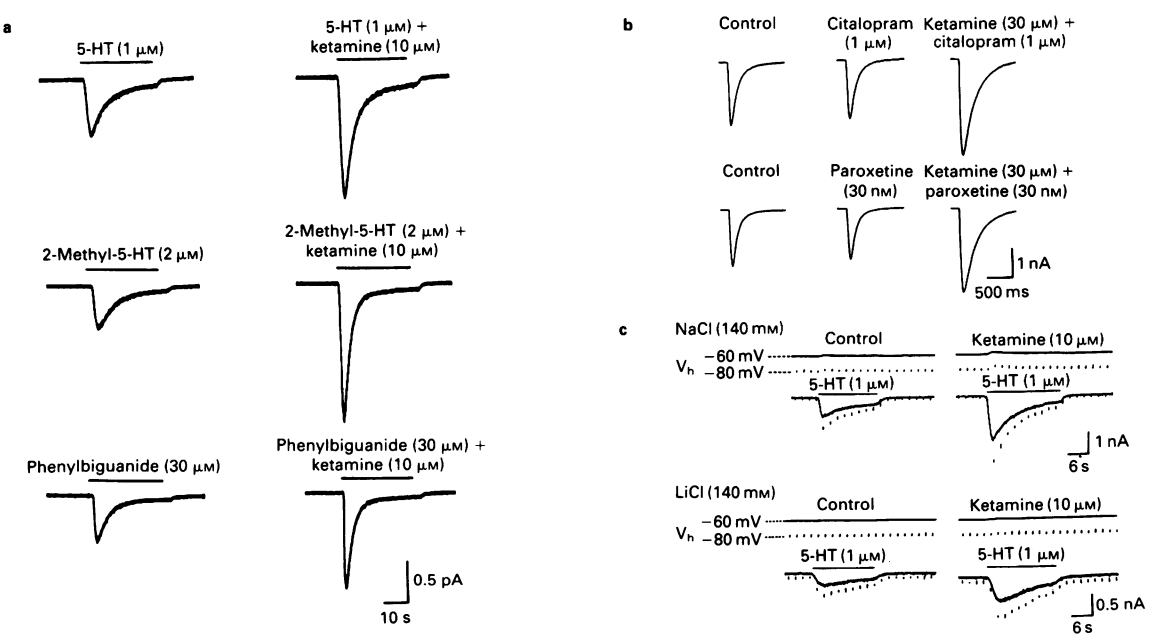

Figure 2 The action of ketamine is not mediated by inhibition of Na^+-dependent 5-hydroxytryptamine (5-HT) uptake. (a) Traces depicting the potentiation by ketamine (10 μM) of inward current responses to 5-HT (1 μM), 2-methyl-5-HT (3 μM) and 1-phenylbiguanide (30 μM) applied by rapid superfusion. All currents were recorded from the same neurone which was voltage-clamped at a holding potential of −60 mV. (b) Traces depicting responses to pressure applied 5-HT (10 μM, 1.4×10^5 Pa, 0.1 Hz, 12 ms duration) in control conditions and following the superfusion of citalopram (1 μM) or paroxetine (30 nM) for 5 min. Note that the uptake inhibitors neither enhance 5-HT-evoked currents, nor prevent the potentiation occurring to ketamine applied in their presence. Each trace is the computer-generated average of 4 responses to 5-HT recorded at a holding potential of −60 mV. (c) Replacement of extracellular Na^+ by Li^+ does not prevent ketamine-induced potentiation of responses to rapidly superfused 5-HT. The upper of each pair of traces represent holding potential (V_h), which was transiently stepped from −60 mV to −80 mV for 100 ms at a rate of 0.5 Hz. Lower traces depict membrane current in response to 5-HT and the voltage steps. Note that, in extracellular media containing either Na^+ or Li^+ as the predominant cation, ketamine (10 μM) potentiates both the amplitude of the 5-HT-evoked current and the increase in membrane conductance which underlies the response.

Ketamine, at concentrations similar to those used here, blocks the uptake of 5-HT into rat brain synaptosomes and inhibits the binding of the 5-HT uptake inhibitor, paroxetine, to rat brain membranes (Martin *et al.*, 1990). However, in our experiments the 5-HT uptake blockers, citalopram and paroxetine, did not enhance the 5-HT-induced current or prevent its potentiation by ketamine. The neuronal uptake of 5-HT is a sodium-dependent process which cannot be supported by lithium (Wood, 1987). However, ketamine was equieffective in potentiating 5-HT-induced currents in a sodium or lithium based extracellular solution. Collectively, these observations suggest that an inhibition of 5-HT uptake is unlikely to explain the effects of ketamine reported here. The molecular mechanism of the ketamine-induced potentiation is as yet unknown. The 5-HT_3 receptor-mediated current in rabbit nodose ganglion neurones desensitizes rapidly, and the method by which known concentrations of agonist were applied in the present study is likely to result in some degree of desensitization occurring prior to the attainment of a peak current response. In view of this complication, no attempt was made to examine the influence of ketamine upon the 5-HT concentration-effect relationship and we are unable to distinguish between the enhancement of the maximal effect of 5-HT, or an enhancement of its potency, as potential mechanisms underlying the action of ketamine. Clearly, the possibility that ketamine binds to a distinct site on the 5-HT_3 receptor protein to enhance the interaction of agonist and receptor, in a manner analogous to positive allosteric modulators at other ligand-gated ion channels, requires further investigation.

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References

IKEDA, S.R., SCHOFIELD, G.G. & WEIGHT, F.F. (1986). Na^+ and Ca^{2+} currents of acutely isolated adult rat nodose ganglion cells. *J. Neurophysiol.*, **55**, 527–539.

MALEQUE, M.A., WARNICK, J.E. & ALBUQUERQUE, E.X. (1981). The mechanism and site of action of ketamine on skeletal muscle. *J. Pharmacol. Exp. Ther.*, **219**, 638–645.

MACDONALD, J.F., MILJKOVIC, Z. & PENNEFATHER, P. (1987). Use-dependent block of excitatory amino acid currents in cultured neurones by ketamine. *J. Neurophysiol.*, **58**, 251–266.

MARTIN, D.C., INTRONA, R.P. & ARONSTAM, R.S. (1990). Inhibition of neuronal 5-HT uptake by ketamine, but not halothane, involves disruption of substrate recognition by the transporter. *Neurosci. Lett.*, **112**, 99–103.

PETERS, J.A., LAMBERT, J.J. & MALONE, H.M. (1991). Physiological and pharmacological aspects of 5-HT_3 receptor function. In *Aspects of Synaptic Transmission.* ed. Stone, T. London: Taylor & Francis (in press).

WOOD, M.D. (1987). Examination of the relationship between the uptake site for 5-hydroxytryptamine and the high affinity binding site for [^{3}H] imipramine II. The role of sodium ions. *Neuropharmacology*, **26**, 1081–1085.

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