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Effects of neuropeptide Y and agonists selective for neuropeptide Y receptor sub-types on arterioles of the guinea-pig small intestine and the rat brain

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1 The actions of neuropeptide Y (NPY) and agonists selective for NPY receptor subtypes were examined on arterioles from the guinea-pig small intestine and the rat pia in order to characterize the receptors mediating the vasoconstrictor and potentiating effects of NPY.

2 A method was developed for measuring the potentiating effects of NPY in situations where it was not possible to obtain a full concentration-response relationship for the vasoconstrictor. NPY, 50 nM, had a greater potentiating effect on the guinea-pig intestinal arterioles than those from the rat pia.

3 NPY and the Y_1-selective agonist, NPY[Leu^{31},Pro^{34}], potentiated the constrictor responses to U46619 in both arterioles and responses to noradrenaline in the guinea-pig arterioles. There was marked desensitization of the potentiating effect, and cross-desensitization between NPY and NPY[Leu^{31},Pro^{34}]. Both NPY and NPY[Leu^{31},Pro^{34}] caused constriction of the rat pial arterioles but not of those from the guinea-pig intestine.

4 The Y_2-selective agonist PYY(13–36) caused no potentiation or vasoconstriction and did not affect the potentiation by NPY or NPY[Leu^{31},Pro^{34}].

5 The potentiating and vasoconstrictor effects of NPY on these arterioles were mediated by Y_1 receptors.

Keywords: Vascular smooth muscle; arteriole; neuropeptide Y receptors; NPY[Leu^{31},Pro^{34}]; PYY(13–36)

Introduction

Neuropeptide Y (NPY) is a 36-amino-acid peptide that is found in a variety of central and peripheral neurones. It occurs in sympathetic nerves supplying the heart and blood vessels, and this has led several investigators to consider its possible role in cardiovascular regulation. NPY injected into the systemic circulation produces a moderate but prolonged rise in arterial blood pressure but the normal plasma level of NPY is low (Morris *et al.*, 1986; 1987; Corder *et al.*, 1988) and it seems unlikely that it functions as a circulating hormone (Pernow *et al.*, 1987). When it is applied to isolated blood vessels the most prominent action of NPY is the potentiation of the effects of a variety of vasoconstrictor substances or vasoconstrictor nerve stimulation. NPY itself will also cause vasoconstriction in some vessels but the effect if often small and requires higher concentrations of NPY than are needed to produce potentiation (Morris & Murphy, 1988; Abel & Han, 1989). In at least one arteriole, NPY causes potentiation at nanomolar concentrations, but negligible constriction in concentrations up to 1μM (Neild & Kotecha, 1990). The other major peripheral action of NPY is on nerve terminals, where it reduces neurotransmitter release (Lundberg & Stjarne, 1984; Potter, 1984). The physiological importance of this action was most clearly demonstrated in the dog heart, where NPY from sympathetic nerves reduced acetylcholine release from the vagus (Potter *et al.*, 1989).

There is now clear evidence that there are at least two types of neuropeptide Y receptor (Wahlestedt & Hakanson, 1986; Wahlestedt *et al.*, 1990) termed Y_1 and Y_2. Y_1 receptors appear to be present on many types of vascular smooth muscle, where they mediate muscle contraction and the potentiation of the vasoconstrictor effects of other substances. Y_2 receptors are found mainly on neuronal tissue, including sympathetic nerves supplying blood vessels, where they modulate neurotransmitter release. The different NPY receptors can be activated selectively by certain NPY analogues. Modifications of the amino acid sequence near the C-terminal end of the NPY molecule has produced Y_1-selective agonists (Fuhlendorff *et al.*, 1990); removal of amino acids from the N-terminal end of the molecule produces a varying degree of selectivity for Y_2 receptors (Potter *et al.*, 1989; Michel *et al.*, 1990).

When injected systemically Y_1 agonists cause a rise in blood pressure similar to that caused by NPY and this would be expected from their vasoconstrictor and potentiating effects on vascular smooth muscle. Y_2 agonists also produce a small rise in blood pressure, but this is the opposite of what would be expected if their action were to reduce neurotransmitter release from nerves. By reducing sympathetic neurotransmitter output they should cause a fall in peripheral resistance. Y_2-selective agonists do have some action on Y_1 receptors which could result in vasoconstriction, but at least 100 fold higher concentrations are required to obtain effects comparable to those of NPY (Wahlestedt & Hakanson, 1986; Rioux *et al.*, 1986; Modin *et al.*, 1991) or Y_1-selective analogues (Schwartz *et al.*, 1989), and it seems unlikely that this could account for their pressor action. Another possibility is that there are significant numbers of Y_2 receptors on vascular smooth muscle in some tissues which mediate constriction or potentiation. Although on larger arteries the effects of NPY are mediated by Y_1 receptors, Wahlestedt *et al.* (1990) have drawn attention to a possible parallel with the α-adrenoceptor system, where the predominantly presynaptic α_2 receptors are found to mediate smooth muscle constriction in some arteries and particularly in the smaller arterioles (Faber, 1988). As the small arteries and arterioles are the region where the nervous system exerts its major influence on circulatory control mechanisms, this is where nerve-released NPY will have its main physiological effect. In the pig,

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Modin et al. (1991) have shown vasoconstrictor effects in the spleen which seem to be mediated by Y_2 receptors.

The experiments in this paper were performed to see if Y_2 receptors mediating vasoconstriction or potentiation could be found on arteriolar smooth muscle. We chose two types of arteriole that showed different responses to NPY and which might differ in the types of NPY receptors that they possessed. Arterioles from the submucosa of the guinea-pig small intestine show only the potentiating effect of NPY, with no effect on smooth muscle membrane potential and no direct constrictor effect. Arterioles in the pia of the rat brain show potentiation, constriction, and smooth muscle depolarization, and might have had multiple NPY receptor types. Our results, however, show that all these effects in both arterioles are mediated by Y_1 receptors.

Methods

Sheets of connective tissue containing arterioles were dissected from the guinea-pig small intestine or the rat brain. Guinea-pigs (Monash outbred strain) of either sex weighing 200–300 kg were used. They were killed by a heavy blow to the head followed by exsanguination by section of the jugular veins, and a piece of ileum was removed. It was cut open and the submucous connective tissue layer removed by first peeling off the mucosa and then peeling the submucous layer from the underlying muscle. Rats (Wistar, 250–300 g) were given an intraperitoneal injection of sodium pentobarbitone (40 mg kg^{-1}) sufficient to induce deep surgical anaesthesia and exsanguinated by section of the abdominal aorta and vena cava. The brain was removed and the pial connective tissue containing the middle cerebral artery and its branches was gently dissected free.

The connective tissue sheet was pinned to transparent silicone rubber on the base of a small chamber (volume 1.0 ml) mounted on an inverted compound microscope, and continuously superfused with warmed oxygenated physiological saline at 6.0 ml min^{-1}. The saline contained (mmol l^{-1}): Na^+ 146, K^+ 5, Ca^{2+} 2.5, Mg^{2+} 2, Cl^- 134, HCO_3^- 25, $H_2PO_4^-$ 1 and glucose 11, and was equilibrated with 95% O_2/5% CO_2. The temperature in the chamber was 30°C. High potassium solution for determining maximal arteriolar constriction (Neild & Kotecha, 1989) was made by replacing 95 mM of Na^+ with K^+ to give a K^+ concentration of 100 mM.

Vasoconstrictor drugs were applied to the tissue from a micropipette placed within 50 μM of the arteriole. Noradrenaline was ejected by ionophoresis; U46619 was ejected from lower resistance pipettes by pressure. The duration of the ejection pulse and the position of the pipette were changed to grade the size of the constrictor response. NPY and its analogues were applied by adding them to the superfusion solution to produce a known concentration.

The diameter of the arterioles was monitored by computer analysis of a television image from a camera attached to the microscope (Neild, 1989).

Catecholamine-containing nerves were demonstrated histochemically by the FAGLU method of Furness et al. (1977).

Drugs used were: noradrenaline bitartrate (Sigma), 9,11-dideoxy-9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$ (U46619, Cayman Chemical Co), neuropeptide Y (porcine sequence, synthesized by Monash University Department of Biochemistry), NPY[Leu^{31},Pro^{34}], PYY(13–36) (Auspep, Melbourne).

A quantitative indication of the potentiating effect of NPY or its analogues was required for this study. We could not use a method based on concentration-response curves for NPY or vasoconstrictors, as these arterioles showed marked tachyphylaxis and desensitization to noradrenaline, U46619, NPY, and combinations of these. Concentration-response relationships could only be obtained by use of single concentrations at intervals of at least 20 min. A method for measuring potentiation was devised, and is explained in the Appendix. A parameter P was calculated as an index of the potentiating effect; values greater than 1 indicated potentiation. The mean of P and its standard error were calculated for particular combinations of vasoconstrictor and potentiator, and significance of differences between Ps was determined using Student's t test, with $P<0.05$ taken to indicate significance. P values in the text are given ± the standard error of the mean.

Results

Arterioles from the guinea-pig small intestine

Noradrenaline (NA) was applied to the arteriole by ionophoresis from a micropipette every 5 min, and it produced a brief constriction of consistent amplitude as shown in Figure 1. When 50 nM NPY was added to the superfusing solution starting 2 min before an application of NA, the response to NA was increased. From 23 experiments the mean value of P for this potentiating effect of NPY was 4.74 ± 0.40. The NPY was left in contact with the arteriole for a total of 7 min, and it can be seen that the potentiating effect declined, so that the second response to NA in the presence of NPY was smaller than the first. In addition to this tachyphylaxis there was a profound desensitization to NPY, such that a second application of NPY 18 min after the end of the first application produced significantly less potentiation (P = 2.22 ± 0.40, $n = 8$) than the first. The results from these and similar experiments using receptor-specific agonists are summarized in Table 1.

An agonist selective for NPY Y_1 receptors (Fuhlendorff et al., 1990), NPY[Leu^{31},Pro^{34}], had very similar effects to NPY. Exposure of the arteriole to 50 nM NPY[Leu^{31},Pro^{34}] potentiated the response to NA (P = 6.65 ± 1.27, $n = 8$) and the

Table 1 Potentiation of responses of guinea-pig intestinal arterioles to noradrenaline (NA) by neuropeptide Y (NPY), the Y_1-selective agonist NPY[Leu^{31},Pro^{34}] and the Y_2-selective agonist PYY(13–36)

Substance applied first	P	*Substance applied second 18 min after first*	P	*Significant reduction of second response?*
NPY	4.74 ± 0.40 $n = 23$	NPY	2.22 ± 0.40 $n = 8$	Yes
		Y_1ag	3.93 ± 0.46 $n = 5$	No
Y_1ag	6.65 ± 1.27 $n = 8$	Y_1ag	2.51 ± 0.51 $n = 6$	Yes
		NPY	1.86 ± 0.14 $n = 5$	Yes
Y_2ag	1.06 ± 0.06 $n = 7$			
		NPY	6.65 ± 0.84 $n = 5$	No

Data from experiments using the same protocol as that illustrated in Figure 1.
Y_1ag = NPY[Leu^{31},Pro^{34}]; Y_2ag = PYY(13–36).

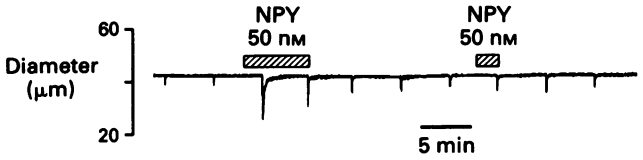

Figure 1 Effect of 50 nM neuropeptide Y (NPY) on the contractile responses caused by brief applications of noradrenaline (NA) to an arteriole from the guinea-pig small intestine. NA was applied to the arteriole every 5 min by ionophoresis from a micropipette. The duration of the ionophoretic current was 1 s, and the resulting constriction shows as a brief downward deflection on the diameter record. The initial exposure to NPY for 2 min before a pulse of NA caused an increase in the amplitude of the constriction, but a subsequent application of NPY 18 min after the first had a much smaller effect.

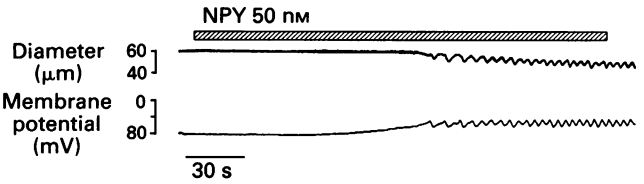

Figure 2 Effects of 50 nM neuropeptide Y (NPY) on the diameter and smooth muscle membrane potential of an arteriole from the rat pia. NPY caused a constriction and depolarization that developed after a delay of about 60 s. There were oscillations of both membrane potential and diameter, with the peaks of depolarization preceding the peaks of contraction.

response to a second exposure to NPY[Leu^{31},Pro^{34}] 18 min later was significantly reduced ($P = 2.51 \pm 0.51$, $n = 6$). The initial potentiating effect ($P = 6.65$) was not significantly different from the effect of the same concentration of NPY ($P = 4.74$). There was interaction between the effects of NPY and NPY[Leu^{31},Pro^{34}]. Prior exposure of the artery to 50 nM NPY[Leu^{31},Pro^{34}] significantly reduced the P value for the potentiation caused by a subsequent application of NPY; 50 nM NPY reduced the potentiating effect of NPY[Leu^{31}, Pro^{34}] but the reduction of P was not significant.

The similarity of the effects of NPY and NPY[Leu^{31},Pro^{34}] and their interaction strongly suggest that they were acting via the same receptors and internal chemical pathways to cause their potentiating effect.

An agonist selective for Y_2 receptors (Wahlestedt & Hakanson, 1986), 50 nM PYY(13–36), caused no potentiation ($P = 1.06 \pm 0.06$, $n = 7$, not significantly different from 1), and did not reduce the potentiating effect of a subsequent application of 50 nM NPY 18 min later ($P = 6.56 \pm 0.84$, $n = 5$). Higher concentrations of PYY(13–36) were tried in a few experiments, but were also without effect (100 nM, $n = 2$; 200 nM $n = 1$).

In all the experiments described above there was no evidence of constriction caused by NPY, NPY[Leu^{31},Pro^{34}], or PYY(13–36) alone.

Arterioles from the rat brain

NPY 50 nM applied to the arterioles in the isolated pial connective tissue of the rat brain produced depolarization of the arteriolar smooth muscle and constriction of the arteriole. These effects developed slowly after a delay of approximately 2 min, as shown in Figure 2. The mean constriction was $20.2 \pm 6.47\%$ of the maximum, and the membrane potential changed from a mean of -53 ± 0.94 mV to -39.5 ± 0.75 mV ($n = 8$). Similar effects were obtained with NPY-[Leu^{31},Pro^{34}], but PYY(13–36) caused no constriction or depolarization in concentrations up to 1 μM. The constriction and depolarization caused by 100 nM NPY (constriction 24.2 ± 3.60, membrane potential from -52.0 ± 0.81 to -38.4 ± 2.14; $n = 7$) were not significantly greater than those caused by 50 nM.

The constriction caused by NPY complicated the analysis of the potentiating effects, so subsequent experiments were performed using 12.5 nM NPY, which did not cause arteriolar constriction in this preparation. The stable thromboxane analogue U46619 was used as a vasoconstrictor, as these arterioles do not constrict in response to NA. The constrictor responses were complex, consisting of an initial rapid constriction followed by a slower component with superimposed oscillations (Figure 3). Measurements were made on the initial component, as this part of the response closely resembled the responses obtained from the guinea-pig small intestine arterioles.

As shown in Figure 3, both NPY ($P = 2.44 \pm 0.26$, $n = 10$) and NPY[Leu^{31},Pro^{34}] ($P = 2.67 \pm 0.31$, $n = 7$) caused

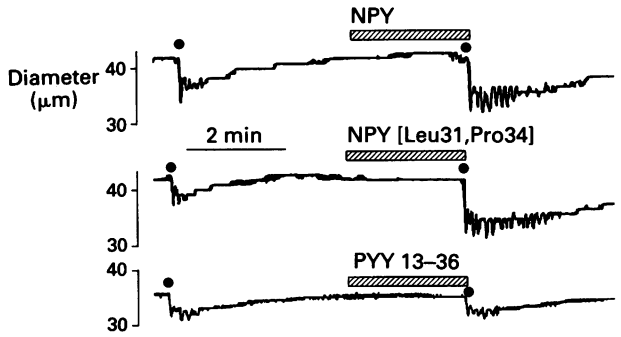

Figure 3 Effects of neuropeptide Y (NPY), NPY[Leu^{31},Pro^{34}], PYY(13–36) on the contractile responses of the rat pial arterioles to U46619. U46619 was applied as a 1 s pulse from a micropipette at the times marked by (●). NPY and NPY[Leu^{31}, Pro^{34}] caused significant potentiation, but PYY(13–36) did not. Traces from 3 different arterioles.

significant potentiation of the vasoconstrictor responses, whereas PYY(13–36) had no effect ($P = 1.02 \pm 0.02$, $n = 5$). Three experiments with 25 nM PYY(13–36) still produced no evidence of a potentiating effect ($P = 1.09 \pm 0.04$, $n = 3$), nor did single experiments with concentrations of 50 nM, 500 nM, and 1 μM.

Comparison of the effects of neuropeptide Y on the two types of arteriole

In order to compare the potentiating effect of NPY on the two types of arteriole a series of experiments were conducted on the guinea-pig intestinal arterioles using the same protocol that was used for the rat pial arterioles i.e. U46619 as the vasoconstrictor, and 12.5 nM NPY. Under these conditions P for the potentiating effect of NPY in the guinea-pig intestinal arterioles was 4.92 ± 1.10, $n = 7$, which was significantly greater than the value of 2.44 found for the rat arterioles. We conclude therefore that the guinea-pig intestinal arterioles are more sensitive to NPY than the rat pial arterioles.

Previous studies on the innervation of the rat pial arteriole have shown that the sympathetic innervation of these vessels does not extend to all the arterioles in our preparation (Hill *et al.*, 1986). In contrast, all the arterioles in our intestinal preparation receive a sympathetic innervation. Furthermore, the sympathetic innervation density is at least three times higher in the intestinal arterioles (Neild, 1984) than in the most densely innervated pial arterioles (Hill *et al.*, 1986). As the sympathetic nerves contain NPY, we thought it possible that their presence may influence the sensitivity of the arterioles. We therefore examined the effects of NPY on 17 rat pial arterioles that were checked after the experiment for the presence of catecholamine-containing nerves. The potentiating effect of 12.5 nM NPY on the constriction to U46619 did not depend on the presence of sympathetic nerves; P for innervated arterioles was 2.1 ± 0.19, $n = 8$, and P for non-innervated arterioles was 2.85 ± 0.87, $n = 9$.

Discussion

Our results show that in both the guinea-pig intestinal arterioles and the rat pial arterioles the potentiating effect of NPY was mediated entirely by Y_1 receptors. NPY[Leu^{31}, Pro^{34}], a well characterized Y_1 agonist with very little activity at Y_2 receptors (Fuhlendorf *et al.*, 1990), was as effective as NPY in producing potentiation of responses to NA or U46619. The lack of effect of PYY(13–36) showed not only that there were no Y_2 receptors involved but also that the concentrations used did not activate Y_1 receptors in this tissue. Although it is generally agreed that NPY analogues based on shortened C-terminal sequences show some selectivity for Y_2 over Y_1 receptors (Wahlestedt *et al.*, 1990), there have been suggestions that they sometimes showed significant activity at Y_1 receptors also. In particular, they can raise blood pressure in anaesthetized rats (Potter *et al.*, 1989), whereas a compound acting only on Y_2 receptors would be expected to lower blood pressure by reducing neurotransmitter release from perivascular sympathetic nerves. The hypertensive effect of short C-terminal is perhaps therefore due to Y_2-mediated vasoconstriction by an action on the arteriolar muscle. The coronary vessels show some vasoconstriction in response to high concentrations of NPY(16–36) and NPY(19–36) (Rioux *et al.*, 1986), but this alone would be insufficient to produce the observed hypertensive effects. We feel our studies make it unlikely that the intestinal or cerebral vascular beds are involved, unless there is marked heterogeneity of responses in different regions of these tissues.

The magnitude of the potentiating effect of 12.5 nM NPY when U46619 was the vasoconstrictor in the guinea-pig intestinal (P = 4.92) and rat pial (P = 2.44) arterioles was different. We have not been able to discern the reason for this difference, other than to show that it is not due simply to differences in the sympathetic innervation, as the sensitivity of the pial arterioles to NPY was the same in sympathetically innervated and non-innervated vessels. However, regions that did not receive a sympathetic innervation were probably still innervated by parasympathetic NPY-containing nerves from the pterygopalatine ganglion (Cavanagh *et al.*, 1990). These nerves also contain vasoactive intestinal polypeptide (VIP) and are probably vasodilator. The function of the NPY in them is not known; in the uterine artery NPY reduces the vasodilator effect of VIP (Morris, 1990).

The variation in sensitivity to NPY in different vascular beds may be related to different sources of endogenous ligand. It is usually assumed that in arteries, NPY released from the sympathetic nerves acts as a co-transmitter, and this is probably the case for the intestinal arterioles that we have studied. However, the finding of high levels of NPY in the cerebrospinal fluid of rabbits has led to the suggestion that NPY in cerebrospinal fluid may be a modulator of cerebral vascular tone (McDonald *et al.*, 1988). Our finding that the sensitivity of smooth muscle in the pial arterioles to NPY is independent of their sympathetic innervation is compatible with that view.

Appendix

Comparison of the magnitude of potentiating effects–T.O. Neild

It is generally agreed that potentiation (also called synergy) is best detected and quantitated by experiments which determine iso-effective combinations of the interacting substances (Berenbaum, 1989). In our case this was not possible, because NPY alone caused no constriction of the intestinal arterioles. It was therefore obvious that the effect of NPY was synergistic rather than additive, but the ideal analysis by determining iso-effective combinations and plotting isoboles could not be carried out. We have, therefore, developed a new method of analysis that enabled us to quantitate the potentiating effect of NPY on transient constrictions caused by brief applications of vasoconstrictors. Unlike the isobole method, it cannot distinguish a potentiating effect from an additive effect when the interacting substances both cause constriction of the arteriole. It uses an arbitrary equation to derive an index which increases with increasing potentiation and can be used to detect quantitative differences in potentiation.

Individual arterioles were stimulated with a constant constrictor stimulus at regular intervals. The amplitude of the constriction was measured in control conditions (c) and in the presence of one concentration of NPY (n). The maximum constriction (max) that the arteriole could produce was also measured, and used to normalize data from different experiments. The amount of vasoconstrictor applied was varied between experiments to give a range of control response amplitudes; the smaller control responses were increased more than larger responses that were closer to the maximum. If the data were plotted with normalized control response amplitude on the abscissa scale and the ratio of potentiated to control amplitude on the ordinate scale, the points fell around a curve as shown in Figure 4. Data from all types of experiment conformed to this pattern, but where the potentiating effect was greater the curve intersected the ordinate at a higher value.

An equation was found (equation 2) which produced a curve that matched the distribution of the data points and contained only 1 free parameter. It was derived from the expression:

$$P = \left(\frac{n}{max-n}\right) / \left(\frac{c}{max-c}\right) \qquad \text{equation 1}$$

We have used this expression to calculate the parameter P for use as a quantitative index of the potentiating effect. A value of P applies to one concentration of the potentiating substance and the whole range of concentrations of the vasoconstrictor, e.g. P for 50 nM NPY and noradrenaline was 4.74. Differences in P between experimental situations indicate differences in the magnitude of the potentiating effect;

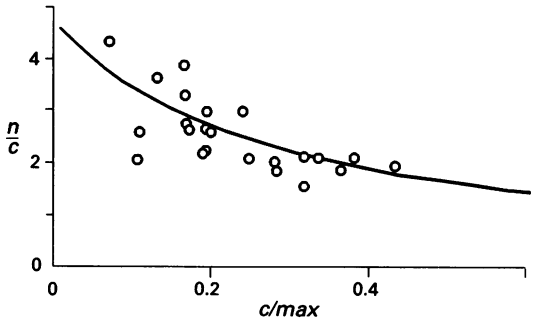

Figure 4 Plot of the ratio of potentiated (n) to control (c) responses against normalized control response amplitude. These data were from experiments using noradrenaline as the vasoconstrictor, with potentiation caused by 50 nM neuropeptide Y (NPY). A trace from one of these experiments is shown in Figure 1. The curve was plotted using equation 1 with a value of 4.74 for P. This value of P was found by taking the mean of individual values calculated from each experiment; it was not derived by fitting the curve to the points shown in this graph.

a value of 1 indicates no potentiation, with higher values for greater potentiating effect.

Re-arranging equation 1 gives:

$$\frac{n}{c} = \frac{max}{c + \frac{(max-c)}{P}} \qquad \text{equation 2}$$

which was used to produce the curve in Figure 4. The limit of this expression as c approaches max is 1, as would be expected intuitively. A control response that is already maximum cannot be increased in amplitude, no matter how great the potentiating effect. As c approaches 0 the expression approaches P, showing that P indicates the greatest factor by which a particular potentiating influence can increase a response. Control responses of intermediate size will be increased by some factor less than P, depending on their size.

In practice we prefer to find the mean value of P for a particular set of data by calculating the mean of individual values calculated for each experiment rather than by finding the best fit of the curve

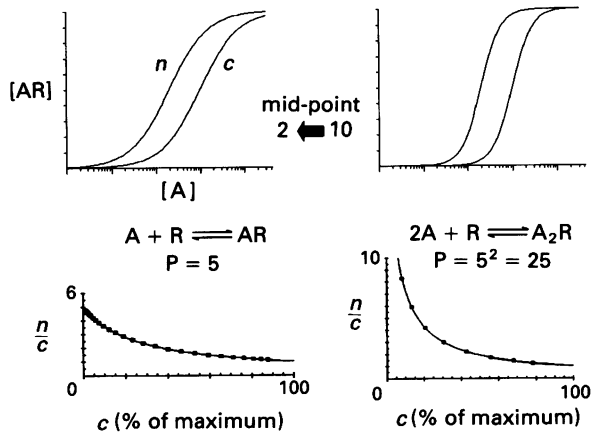

Figure 5 Simulation to show the P values obtained from the analysis of a situation in which the potentiating mechanism shifts the stimulus-response curve to the left (upper plots). A simple logistic function was used to calculate sigmoid curves corresponding to first order (left) and second order (right) binding reactions. Concentration of the agonist [A] is in arbitrary units; potentiation was represented by shifting the mid-point of the curves from 10 units to 2. The lower plots of n/c against c are the type that would be used to analyse experimental data, as shown in Figure 4. The simulated data points taken from the sigmoid curves fit exactly to a curve drawn using equation 2 with P equal to the ratio of the midpoints of the sigmoid curves raised to the power of the order of reaction.

given by equation 2 to the whole data set. Both sides of equation 2 contain terms that are subject to experimental error, and a true best fit with an estimate of the variance of P cannot be easily obtained. When P is calculated from the mean of values from individual experiments standard statistical methods can be used to test for differences between Ps and between P and 1.

The application of this analysis to simulated situations in which a graph of log (stimulus) and response was sigmoidal, and potentiation shifted this graph to the left, as shown in Figure 5. The stimulus-response curves were calculated from logistic equations for the fraction of binding sites occupied by various concentrations of a ligand. 'Control values' were taken from one curve and 'potentiated values' taken from the shifted curve at the same 'stimulus' value. P was calculated for each pair of values using equation 1, as would be done for experimental data, and the mean value of P was used to plot a curve through the measured points. The results using first and second order binding reactions are shown in Figure 5.

These simulations show that a value of P obtained experimentally is related to a shift in the stimulus-response relationship, even though the full relationship had not been obtained. In the case of a first order system, P would be the ratio of mid-points of the two curves; in the case of higher order systems P would be equal to this ratio raised to the power of the order.

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