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PRESYNAPTIC α-ADRENOCEPTOR BLOCKING PROPERTIES AMONG TRI- AND TETRA-CYCLIC ANTIDEPRESSANT DRUGS

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1 The effect of various antidepressants (5×10^{-8} to 2×10^{-5} M) on the resting overflow of tritium, on the evoked overflow and the contractile response to electrical stimulation (2.5 Hz, 2.0 ms) has been determined in mouse vas deferens previously incubated with [^{3}H]-(−)-noradrenaline.

2 Mianserin and ORG GC 94 produced a concentration-dependent increase of more than two fold in the electrically evoked overflow and the contractile response and, at the highest concentration, slightly increased resting release. These effects were largely unchanged in the presence of a concentration of cocaine effective in blocking noradrenaline uptake (1.1×10^{-5} M).

3 The ability of phentolamine (1×10^{-5} M) to increase both the evoked overflow of tritium and the contractile response was greatly reduced when these parameters were already elevated by the presence of mianserin or ORG GC 94.

4 The inhibitory effect of exogenous (−)-noradrenaline on evoked overflow was greatly reduced in the presence of mianserin or ORG GC 94 (4×10^{-6} M).

5 The inhibitory effect of clonidine on the twitch response of the mouse vas deferens was antagonized by mianserin and ORG GC 94 in a competitive manner (pA_2 values 7.3 and 7.1 respectively).

6 Maprotiline, desipramine and nortriptyline ($>3 \times 10^{-6}$ M) produced a parallel fall in both evoked tritium overflow and in the contractile response and increased the resting overflow at higher concentrations. These effects were largely unchanged in the presence of cocaine (1.1×10^{-5} M).

7 Doxepin, imipramine and iprindole all increased resting overflow at high concentrations (2×10^{-5} M) but produced only small changes in evoked overflow and in the contractile response at lower concentrations.

8 It is concluded that mianserin and ORG GC 94 produce a blockade of presynaptic α-adrenoceptors which could contribute to an antidepressant effect but that this type of action is not common to all antidepressants.

Introduction

Many tri- and tetra-cyclic antidepressants are able to inhibit the re-uptake of noradrenaline and therefore probably raise the concentration of noradrenaline in the synaptic cleft. Such a mechanism may explain the antidepressant action of these drugs (Schildkraut, 1965) but the relationship between clinical effect and ability to inhibit noradrenaline uptake is poor (Barth, Manns & Muscholl, 1975; Ghose & Coppen, 1977). However, noradrenaline uptake is not the only factor determining noradrenaline concentrations in the synaptic cleft as noradrenaline release is now known to be influenced by a variety of mechanisms both in peripheral tissues and in the central nervous system (Langer, 1977; Starke, 1977). One of the most important mechanisms controlling noradrenaline release is mediated through presynaptic α-adrenoceptors and it has been shown that in mouse vas deferens, amitriptyline is capable of blocking presynaptic α-adrenoceptors, thus producing a large rise in the tritium released in response to nerve stimulation of tissues previously incubated with [^{3}H]-noradrenaline (Hughes, 1978). Since the possession by antidepressant drugs of a variable degree of blocking activity at presynaptic α-adrenoceptors (which would therefore raise synaptic noradrenaline levels by increasing noradrenaline release) may go some way to explaining the poor relationship between uptake blockade and clinical response to antidepressants, we have investigated a number of other antidepressants for possible blocking activity at presynaptic α-adrenoceptors in the mouse vas deferens.

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Methods

Measurement of resting and evoked tritium overflow and contractile response

The method used was as described by Hughes (1978) except that the ligature at the top of the vas deferens was attached to an isometric transducer (Dynamometer UFI; resting tension 0.5 g) and changes in tension were recorded on a Devices M2 recorder. The tissue was allowed to equilibrate and wash for 60 min and up to 6 periods of electrical stimulation (15 V, 2 ms pulse width, 2.5 Hz, 180 to 220 mA for 45 s) were applied. Additions of unlabelled noradrenaline were made directly into the tissue bath in a volume of 20 μl immediately after a change of bath fluid.

Resting overflow of tritium was taken as the average of the tritium content of the two samples collected immediately before a period of electrical stimulation and evoked overflow was taken as the tritium in the sample surrounding the tissue during a period of stimulation together with the tritium in the succeeding sample in excess of that expected in both samples from the resting overflow. Samples collected during the first period of stimulation (S1) were discarded as they often gave values which were unrepresentative of those found in later periods (S2 to S6). The next period of stimulation (S2) was used as a control period for each tissue and the resting overflow, evoked overflow and contractile response in later periods were expressed as a percentage of these control values for each tissue.

At the end of the experiment the tissue was removed from the tissue bath, wrapped in a small square of Kleenex tissue and combusted in a Packard Tri-Carb Oxidiser (model 305). Recovery of radioactivity from the combustion process was 94.3 ± 0.4% (mean ± s.e. mean; $n = 6$) as determined by combustion of known amounts of [^{3}H]-(−)-noradrenaline on tissue paper. The results from the combusted samples were corrected for this recovery.

Measurement of presynaptic α-adrenoceptor blocking potency

Mouse vasa deferentia were set up as described above except that the physiological saline contained cocaine (1.1×10^{-5} M), the tissue bath volume was 61 ml and that the contractile response to electrical stimulation (0.1 Hz continuously) was recorded with a Statham Gold Cell isometric transducer. Cumulative concentration-response curves for the ability of clonidine to inhibit the twitch response resulting from electrical stimulation were recorded in the absence of mianserin or ORG GC 94 or after 20 min exposure to appropriate concentrations of these drugs. Each concentration of clonidine was allowed to remain in contact with the tissue for 2 min and inhibition was measured as a percentage of the height of the twitch response immediately before the addition of clonidine.

Drugs

L-Ascorbic acid (BDH), clonidine hydrochloride (Boehringer), cocaine hydrochloride B.P., desipramine hydrochloride (Geigy), disodium ethylenediaminetetraacetic acid (BDH), doxepin hydrochloride (Pfizer), ORG GC 94; 1,3,4,14b-tetrahydro-2,7-dimethyl-2*H*-dibenzo[*b*,*f*]pyrazino[1,2-*d*][1,4]oxazepine (Z)-2-butenediate (Organon), imipramine hydrochloride (Geigy), iprindole hydrochloride (Wyeth), maprotiline methane sulphonate (Ciba), mianserin hydrochloride (Organon), (−)-noradrenaline bitartrate (Sigma), nortriptyline hydrochloride (Lilly) and phentolamine mesylate (Ciba) were used. 17β-Oestradiol (Sigma) was dissolved in ethanol (≈ 2 mg/ml) and an appropriate volume of the ethanolic solution was added to the physiological saline. [^{3}H]-(−)-noradrenaline (sp. act. 9.1 Ci/mmol) was obtained from the Radiochemical Centre, Amersham.

Statistical procedures

Where appropriate, results are given as mean ± standard error of the mean (mean ± s.e. mean) and tests for statistical significance utilised Student's *t* test. Mathematical transformations of crude data to percentage of control value involves the possibility that the transformed values will no longer be normally distributed. Where this transformation has been carried out, tests for statistical significance were carried out by non-parametric methods (Mann-Whitney test; see Snedecor & Cochran, 1967).

Results

At the start of the second stimulation period (S2) the tissues contained large amounts of tritium (6.40 ± 0.28 × 10^5 d/min; $n = 43$) and at least part of this variability is accounted for by variations in tissue weight. There was no statistically significant difference ($P > 0.1$) between the mean tritium content of any of the groups of tissues and in untreated tissues the resting overflow of tritium before the second stimulation period averaged 1498 ± 59 d/min per 2 min collecting period. Electrical stimulation induced an evoked overflow of tritium which averaged 2153 ± 109 d/min and the maximum tension developed during the twitch response to electrical stimulation averaged 0.67 ± 0.07 g ($n = 43$).

In the absence of antidepressants, resting overflow, electrically evoked overflow and the contractile response all fell slightly in succeeding stimulation

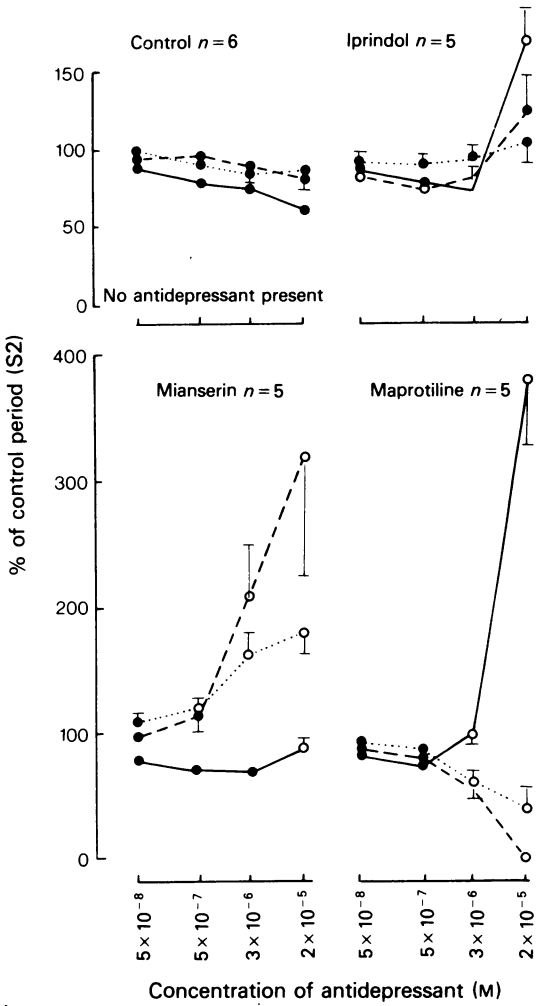

Figure 1 Effect of increasing concentrations of various representative antidepressants on resting tritium overflow (complete line) and on evoked overflow (dotted line) and contractile response (dashed line) induced by electrical stimulation of mouse vas deferens previously incubated with [^{3}H]-(−)-noradrenaline. For each tissue responses in stimulation periods S3 to S6 (in the presence of 5×10^{-8}, 5×10^{-7}, 3×10^{-6} or 2×10^{-5} M concentrations of the appropriate antidepressant) are expressed as a percentage of the response in the control period (S2) before application of antidepressant. The points represent means, the bars show s.e. and the number of experiments contributing to each mean is shown (*n*). Open points show a statistically significant difference ($P < 0.05$; Mann-Whitney test) from corresponding points in tissues not exposed to antidepressants (control group). Standard errors have been omitted where they fall within the area of the points.

periods (S3 to S6) and comparisons between treated and untreated tissues have therefore been made at corresponding times from the start of the experimental procedure and non-parametric statistical methods have been used (Figure 1).

Effects of antidepressants on resting and evoked overflow and on the contractile response

Application of mianserin (Figure 1) or of ORG GC 94 produced a rise in the contractile response and in evoked tritium overflow which was concentration-dependent, while resting tritium overflow was not significantly changed except at the highest concentration (2×10^{-5} M) where a small (<30%) but statistically significant ($P < 0.01$) increase was observed. With all the other antidepressants a very marked rise in resting overflow of 80% to 220% was seen at the highest concentration tested and with maprotiline (Figure 1) and nortriptyline this effect was evident at a lower concentration (3×10^{-6} M) although the magnitude of the effect was much smaller.

In contrast to the increase in contractile response and in evoked tritium overflow which was produced by mianserin and by ORG GC 94, maprotiline (Figure 1) together with desipramine and nortriptyline depressed the contractile response and the evoked tritium overflow in parallel and the profiles for these three drugs were quantitatively very similar. Doxepin produced a fall in the contractile response at low concentrations (5×10^{-7} M) while imipramine had no significant effect and with both drugs evoked overflow rose slightly and then fell at the highest concentration (2×10^{-5} M).

At low concentrations (5×10^{-7} M) iprindole (Figure 1) produced a marginal reduction in the contractile response but this parameter and the evoked tritium overflow then showed a tendency to rise at higher concentrations ($> 3 \times 10^{-6}$ M) although there was considerable variation between tissues with this drug and the latter effect did not achieve statistical significance.

Effects in the presence of cocaine

In the presence of cocaine (1.1×10^{-5} M) the application of mianserin (4×10^{-6} M) or of ORG GC 94 (4×10^{-6} M) still produced a highly significant increase ($P < 0.01$) in both evoked tritium overflow and in the contractile response and produced a marginal rise in resting overflow. Maprotiline (4×10^{-6} M) still reduced evoked overflow, reduced the contractile response and increased resting tritium overflow (Figure 2). In single experiments with the other antidepressants no marked diffferences were noted between the effects seen in the presence of cocaine and those seen in its absence.

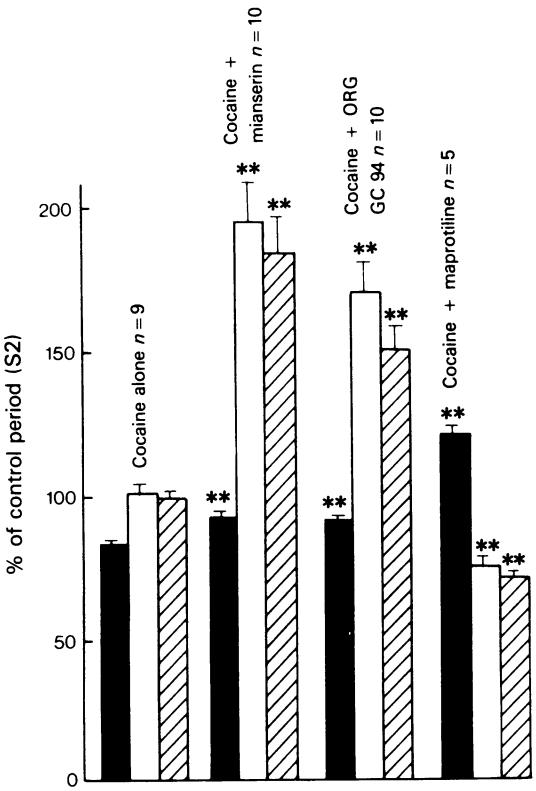

Figure 2 Effects of mianserin, ORG GC 94 or maprotiline (all at 4×10^{-6} M) in the presence of cocaine (1.1×10^{-5} M) on the resting overflow of tritium (solid columns) and on evoked overflow (open columns) and contractile response (hatched columns) induced by electrical stimulation of mouse vas deferens previously incubated with [^{3}H]-(−)-noradrenaline. For each tissue the response has been expressed as a percentage of the response in the control period. Each column represents the mean of a number of experiments (n) and the bars show s.e. **Statistically significant differences ($P < 0.01$; Mann-Whitney test) from the experiments performed in the presence of cocaine alone.

Effects of noradrenaline

In the presence of cocaine (1.1×10^{-5} M) the application of unlabelled exogenous (−)-noradrenaline (0.1 to 1.2 μM) significantly reduced the evoked overflow of tritium and the effect was concentration-dependent (Table 1). The contractile response was also reduced by (−)-noradrenaline but this effect is not reported further since the size of the effect was very variable and quantitation was complicated by a net rise in tissue tone which occurred especially at the highest concentration. In the presence of cocaine and mianserin (4×10^{-6} M), the evoked overflow of tritium was increased to 185.6 ± 20.1% ($n = 5$) of the control overflow and the addition of 0.4 μM (−)-noradrenaline failed to produce any statistically significant reduction in evoked overflow, the corresponding figure in the presence of noradrenaline being 175.6 ± 13.2% ($n = 5$; $P > 0.05$). Similarly, 0.4 μM (−)-noradrenaline failed to produce any significant effect ($P > 0.05$) in the presence of cocaine together with ORG GC 94 (4×10^{-6} M) where the evoked overflow of tritium was 158.2 ± 13.1% while the corresponding figure in the absence of noradrenaline was 161.4 ± 7.1% ($n = 5$).

Effects of phentolamine

In the presence of cocaine (1.1×10^{-5} M), mianserin (4×10^{-6} M) increased the evoked overflow and the contractile response very markedly (Figure 2) and in the presence of these two drugs phentolamine (1×10^{-5} M) produced only a small additional increase in evoked overflow of +4.4 ± 4.4% and in contractile response of +6.8 ± 5.9% ($n = 6$). Similarly, in the presence of cocaine and ORG GC 94 (4×10^{-6} M), phentolamine increased evoked overflow by +28.8 ± 6.0% and the contractile response by +29.0 ± 6.9% ($n = 6$). In the presence of cocaine alone, phentolamine increased evoked overflow by +157.0 ± 13.9% and the contractile response by +95.8 ± 18.8% ($n = 6$) and the difference between the effects of phentolamine in the presence or absence of mianserin (or ORG GC 94) is highly significant statistically ($P < 0.01$).

In single experiments, treatment of tissues with cocaine and phentolamine together (1.1 and 1.0×10^{-5} M respectively) produced the expected large rise in evoked tritium overflow and in the contractile response but the further application of mianserin or ORG GC 94 (4×10^{-6} M) produced a slight reduction in the size of these responses and no additional increase was seen.

Effects of clonidine on the twitch response

In the presence of cocaine (1.1×10^{-5} M) the vas deferens responded to low frequency stimulation (0.1 Hz) with a regular twitch response which averaged 52.8 ± 9.6 mg ($n = 60$) and which was well maintained for at least 2 h. Administration of clonidine (1.5×10^{-9} to 3.6×10^{-8} M) produced a concentration-dependent inhibition of the twitch response which reached equilibrium within 2 min and was reversed by washing the tissue. However, after washing out the clonidine the twitch response became irregular in size and repeated cumulative concentration-response curves could not therefore be obtained from a single tissue. Mianserin and ORG GC 94, as expected, increased the size of the twitch response and

this effect reached equilibrium within 20 min. Inhibition of the twitch response by clonidine was expressed as a percentage of the twitch height before the addition of clonidine and from several determinations of the cumulative concentration-response curve to clonidine in different tissues a mean ED_{50} value was obtained (5.38×10^{-9} M; geometric mean; $n = 11$) and dose-ratios were calculated between this value and the individual ED_{50} values obtained from cumulative concentration-response curves to clonidine, determined in the presence of cocaine together with various concentrations of mianserin or ORG GC 94. The dose-ratios obtained at the various concentrations of mianserin or ORG GC 94 were used to construct $\log_{10}$ (dose ratio − 1) against $\log_{10}$ molar concentration of antagonist plots (Arunlakshana & Schild, 1959) (Figure 3). Regression analysis on the individual values gave a pA_2 value for mianserin of 7.32 ± 0.12 and for ORG GC 94 of 7.14 ± 0.23 (mean ± 95% confidence limits). The slopes of the calculated regression lines were 1.02 ± 0.12 for mianserin and 0.96 ± 0.21 for ORG GC 94 (mean ± 95% confidence limits) which did not differ significantly ($P > 0.6$) from a value of unity which would be expected if the antagonism was competitive. The observation that administration of sufficient clonidine produced effectively 100% inhibition of the twitch response in the presence of all the concentrations of mianserin or ORG GC 94 which were tested also supports the possibility of a competitive character for the antagonism.

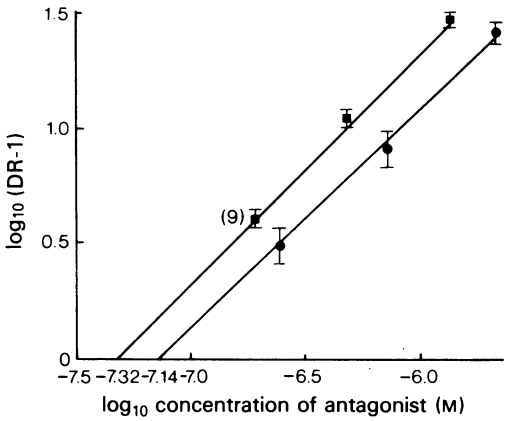

Figure 3 Arunlakshana-Schild plots for the ability of mianserin (■) or ORG GC 94 (●) to antagonize the inhibitory effect of clonidine on the electrically induced twitch response of the mouse vas deferens. The points represent means, each calculated from 8 experiments (unless otherwise shown; figure in parentheses) and the bars show s.e. The regression lines were calculated from the individual values, not the mean values shown in the figure.

Discussion

Tritium overflow from a tissue previously incubated with [^{3}H]-(−)-noradrenaline represents the difference between released tritium and that taken back up into the tissue. The tritium overflowing into the bathing fluid is present as noradrenaline and as metabolic products (Starke, 1977) but the proportion of these metabolic products originally existing as noradrenaline in the synaptic cleft and contributing to the activation of receptors is unknown. No attempt has been

Table 1 Effects of exogenous noradrenaline in the presence of cocaine (1.1×10^{-5} M) on electrically evoked overflow of tritium from mouse vas deferens previously incubated with [^{3}H]-(−)-noradrenaline

Stimulation period	S3	S4	S5
Control tissues (n = 4)			
Evoked overflow (% of control period; S2)	102.2 ± 8.0	91.7 ± 10.1	86.7 ± 11.9
Noradrenaline treated tissues (n = 4)			
Noradrenaline concentration (μM)	0.1	0.4	1.2
Evoked overflow (% of control period; S2)	73.5 ± 1.4	54.7 ± 2.1	36.5 ± 0.9
Difference between treated and untreated tissues	−28.7%	−37.0%	−50.2%
Probability (non parametric test; control tissues versus noradrenaline-treated tissues)	<0.05	<0.01	<0.01

The table shows means ± s.e. means for the evoked overflow of tritium in stimulation periods S3 to S5 expressed as a percentage of that in the control period (S2) for each tissue. Noradrenaline-treated tissues were exposed to exogenous unlabelled (−)-noradrenaline for 15 s before electrical stimulation was applied.

made to separate the tritium into identified molecular species; therefore it must be remembered throughout this work that alterations in tritium overflow may not quantitatively reflect alterations in noradrenaline concentrations in the synaptic cleft.

In the work described in this paper, changes in the resting release and evoked overflow of tritium have been expressed in terms of a percentage of the 'before treatment' values. An alternative method of expression, the use of fractional release or fractional release per shock, has not been employed since we have no evidence that release per shock is constant within a train of pulses or that the relationship between the total tritium content of the tissue and the size of the releasable store remains constant in tissues of different weight or after treatment with different drugs. Furthermore, the use of 'percentage of before treatment' values eliminated the variability in fractional release between tissues which would otherwise be compounded with the variability in response to treatments. Nevertheless, in our experiments the fractional release per shock in control tissues averaged 3.25×10^{-5} which is very close to the value of 3.16×10^{-5} found by Marshall, Nasmyth & Shepperson (1978) though differences in methodology make a comparison between these figures of debatable value.

It has been found previously (Hughes, 1978) that in mouse isolated vas deferens, amitriptyline is capable of blocking presynaptic α-adrenoceptors involved in the control of noradrenaline release and the results described here are compatible with a similar action for mianserin and ORG GC 94. Thus the massive rise in evoked overflow of tritium produced by these two drugs was abolished by phentolamine and cocaine at concentrations capable of completely blocking presynaptic α-adrenoceptors and noradrenaline uptake respectively. Since the increase in evoked overflow was still seen in the presence of cocaine alone, it cannot be due to a reduction in noradrenaline reuptake even though mianserin does block noradrenaline uptake at these concentrations (Harper & Hughes, 1977). The action of phentolamine in enhancing evoked tritium overflow by blockade of presynaptic α-adrenoceptors was greatly reduced in the presence of mianserin or of ORG GC 94 and the ability of exogenous noradrenaline to inhibit evoked overflow by stimulating presynaptic α-adrenoceptors was also inhibited by these drugs. All these effects are compatible with an ability of mianserin and ORG GC 94 to block the presynaptic α-adrenoceptors controlling noradrenaline release and a similar effect has been suggested to occur with mianserin in brain slices (Bauman & Maitre, 1977).

This action was confirmed by the use of clonidine, a relatively selective presynaptic α-adrenoceptor agonist (Starke, 1977) the inhibitory effect of which was antagonized competitively by both mianserin and by ORG GC 94.

Maprotiline, desipramine and nortriptyline all showed similar profiles of activity and reduced both the evoked overflow of tritium and the contractile response in contrast to the effects of mianserin and ORG GC 94. No evidence of blockade of presynaptic α-adrenoceptors was seen, in spite of the known ability of desipramine to block postsynaptic α-adrenoceptors in mouse vas deferens (Hughes, Kneen & Main, 1974) and the close structural similarity of nortriptyline to amitriptyline (which does block presynaptic α-adrenoceptors; Hughes, 1978). The reduction in the contractile response seen at high concentrations could be due to a variety of actions such as a non-specific depression of smooth muscle, a postsynaptic α-adrenoceptor blockade or a local anaesthetic effect. Alternatively, if the increased resting overflow of tritium represents an increase in resting noradrenaline release, this could activate presynaptic α-adrenoceptors thus inhibiting evoked tritium release and the contractile response. This latter mechanism seems unlikely as iprindole raised resting overflow but produced little change or a slight increase in the contractile response. Whatever the mechanisms it is unlikely to be of any therapeutic relevance since the concentrations required (2×10^{-5} M $\approxeq$ 6 mg of free drug per litre) are seldom approached even in the grossest overdose.

The effects of doxepin, imipramine and iprindole were less clear but none of the drugs appeared to show marked presynaptic α-adrenoceptor blocking properties as any rise in evoked overflow was small ($< 30\%$) and blockade of the α-adrenoceptor-mediated control mechanisms usually results in a two to three fold increase in noradrenaline release. Clearly therefore although some antidepressants will produce a blockade of presynaptic α-adrenoceptors this is not a property common to all agents in this group and therefore cannot be a factor in the mechanism of action of this group of drugs as a whole.

The catecholamine theory of depression (Schildkraut, 1965) postulates that an increased synaptic concentration of noradrenaline resulting from blockade of the re-uptake of noradrenaline by antidepressants is involved in the therapeutic effects of these drugs. The clinical efficacy of mianserin and iprindole which do not block noradrenaline reuptake in clinically effective doses (Fann, Davis, Janowsky, Kaurman, Griffith & Oates, 1972; Ghose, Coppen & Turner, 1976) is therefore difficult to reconcile with this hypothesis. The possession of a presynaptic α-adrenoceptor blocking action by mianserin would allow a rise in synaptic noradrenaline levels to take place even in the absence of blockade of reuptake and make the undoubted clinical efficacy of mianserin explainable in terms of the catecholamine theory of

depression. However, no such effect was seen with iprindole which produced little effect on resting overflow, evoked overflow or the contractile response until very high concentrations were reached.

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