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THE EFFECTS OF β-CARBOLINES ON RESPONSES TO ACETYLCHOLINE, NORADRENALINE, 5-HYDROXYTRYPTAMINE AND AMINO ACIDS IN THE RAT SPINAL CORD

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SUMMARY

Various drugs have been applied electrophoretically to Renshaw cells and to unidentified spinal neurones in pentobarbitone anaesthetized or decerebrated rats. Responses to noradrenaline (NA) and 5-hydroxytryptamine (5-HT) have not previously been described at this site and were of two types; either monophasic depression or biphasic depression–excitation. The effect of harmine on these responses was examined. Harmine and harmaline were also tested on the excitant responses to acetylcholine (ACh) and DL-homocysteate (DLH), and on the depressant responses to glycine and γ-aminobutyric acid (GABA).

On some cells harmaline antagonized ACh, but not DLH, and glycine, but not GABA, responses. Harmine caused only non-specific depression and spike configuration changes. The effects of harmine on NA and 5-HT responses were usually non-specific, and any antagonism was usually accompanied by, or soon followed by spike changes. LSD was also tested on the amine responses. LSD itself had a clear depressant effect on neuronal firing rates. It could either antagonize or potentiate NA and 5-HT depressant responses, but the antagonism in particular was closely followed by spike changes. Somewhat more specific antagonism of the late excitation was seen.

INTRODUCTION

The present investigation was performed in an attempt to find an interaction between certain putative transmitters and a group of compounds which induce rhythmical discharges of neurones when ejected electrophoretically into the inferior olivary nucleus of the rat[6,22]. Since olivary neurones alter their discharge frequency

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only marginally following the ejection of some excitatory or inhibitory transmitter candidates[14,21], the usual methods of investigating the actions of electrophoretically applied substances on neuronal firing rates could not be satisfactorily employed in the olive. For this reason we have used spinal interneurones as a test site.

We have tested harmaline and/or harmine, two closely related β-carbolines which cause rhythmical activity in the inferior olive, for any actions on the responses to a number of putative transmitters, namely acetylcholine, L-glutamate, L-aspartate, glycine, γ-aminobutyric acid, noradrenaline and 5-hydroxytryptamine. With the exception of reports on the effects of 5-HT on motoneurone field potentials and on monosynaptic responses[2,3], the actions of the monoamines on spinal neurones in the rat have not previously been described. In addition we have tested the actions of LSD on the responses to these amines.

METHODS

Experiments were performed on 25 male albino rats, 350–450 g. Anaesthesia was induced with pentobarbitone (45 mg/kg) intraperitoneally and was maintained with pentobarbitone administered intravenously from a constant infusion pump. In two animals decerebration was performed under halothane anaesthesia, which was thereafter discontinued.

Details of the lumbar laminectomy, further preparation and methods for recording from spinal cord neurones in the rat have been published previously[5]. Details of most of the drug solutions used were given in a preceding paper[21]. The pH of the monoamine solutions was adjusted to 3.8–4.2. L-Glutamate and L-aspartate were used as 250 m*M* solutions in water, pH 8. Harmaline HCl solutions used were either 100 m*M* in water, 20 m*M* in 140 m*M* NaCl or 10 m*M* in 70 m*M* NaCl; the drug was diluted with NaCl because in early experiments using solutions in water, effects were seen with very low electrophoretic currents. The solution was further diluted because of a tendency of the 20 m*M* in 140 m*M* NaCl solution to crystallize at the low temperature at which the electrodes were stored. The effects of the drug were qualitatively similar whichever solution was used. Harmine was ejected from 70 or 100 m*M* solutions in water of the hydrochloride. The pH of the solutions was not adjusted; for harmaline solutions the pH was in the range 4.6–5.1, and for harmine 4.3–5.0.

Current balancing was not used routinely in these experiments; instead control ejections of Na^+ ions were compared with the effects of depressant compounds (see example in Fig. 2). Compounds were retained by applying 0.5 V across each barrel. Ejecting currents are expressed in nanoamps, nA; where a reduction in the retaining current was sufficient to cause an effect, the residual retain current is expressed as $- \times$ nA.

RESULTS

The effects of β-carbolines on excitant responses

Harmaline was tested on 11 Renshaw cells in 7 rats for any effect on the excitations caused by electrophoretically applied acetylcholine (ACh, 4–32 nA, mean

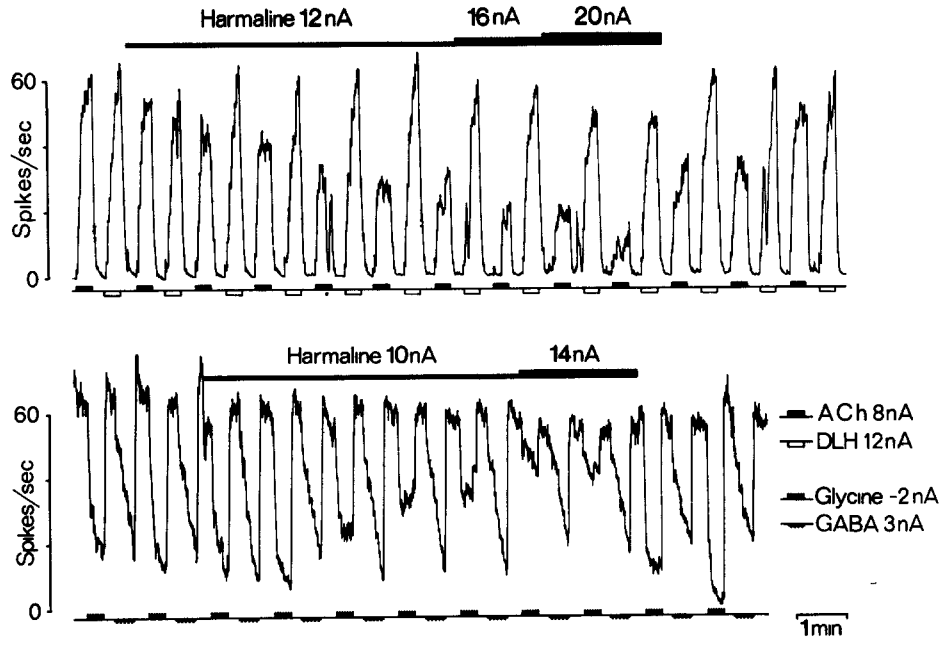

Fig. 1. Action of harmaline on acetylcholine and amino acid responses of a Renshaw cell. Top record: harmaline, 12, 16 and 20 nA was ejected during the cyclical ejections of acetylcholine (ACh) 8 nA and DL-homocysteate (DLH) 12 nA. Harmaline 12 and 16 nA reduced the size of the ACh responses without affecting those to DLH. At 20 nA the DLH responses were slightly reduced. Lower record: harmaline 10 and 14 nA was ejected during the cyclical ejection of glycine — 2 nA and γ-aminobutyric acid (GABA) 3 nA. Glycine responses were reduced considerably more than were the GABA responses. The firing rate of this cell was maintained by the continuous ejection of DLH 5 nA.

15 nA) and DL-homocysteate (DLH, 8–24 nA, mean 15 nA) and on a further 9 cells in 4 rats on ACh (3–60 nA, mean 25 nA), L-glutamate (26–100 nA, mean 47 nA), and L-aspartate (18–70 nA, mean 38 nA). In all cases where ACh and DLH were compared, the ACh responses were reduced or blocked before the responses to DLH were affected. This effect is illustrated in the top ratemeter record of Fig. 1. Harmaline (12, 16 and 20 nA) was ejected during the alternate applications of ACh (8 nA) and DLH (12 nA). It can be seen that harmaline almost wholly blocked the responses to ACh whilst those to DLH were only slightly reduced. Full recovery occurred within 3 min of terminating the harmaline ejection.

In contrast, only 2 of the 9 cells on which ACh was compared with glutamate and aspartate showed specific ACh reduction. With the other 7 cells, all 3 excitants were reduced in parallel.

Harmine was tested on 3 Renshaw cells in 3 rats on the responses to ACh (8–13 nA, mean 10 nA) and DLH (9–15 nA, mean 12 nA) and on a further 10 cells in 4 rats on ACh (1–60 nA, mean 28 nA), L-glutamate (18–100 nA, mean 52 nA) and L-aspartate (18–64 nA, mean 42 nA). In no case was a specific reduction of the ACh excitation observed, although in two cells ACh responses were affected somewhat sooner than those to the amino acids. With the other 11 cells, the responses to all

excitants were reduced in parallel, but in 8 of these cells the action potential height was reduced at the same time as the excitant responses.

The effect of β-carbolines on the responses to inhibitory amino acids

Harmaline was tested on 3 Renshaw cells and on 5 unidentified spinal neurones in 3 rats. Glycine (−2 to 40 nA, mean 12 nA) and GABA, (3–32 nA, mean 10 nA) were ejected alternately so as to cause regular depressions of the DLH-maintained discharge rates of these cells. The effects of harmaline were rather variable. In general no clear effects on the DLH-induced firing rate or on the responses to the depressant amino acids were seen until the spike height was markedly reduced. On only two cells (one Renshaw, one unidentified) was any specific antagonism seen; in these cases the glycine responses were reduced whilst the GABA responses remained relatively unchanged. This is illustrated for the Renshaw cell by the lower ratemeter record in Fig. 1.

Harmine (10–80 nA) was tested on glycine (7–41 nA mean 15 nA) and GABA (3–28 nA, mean 11 nA) responses on 7 unidentified neurones in 3 rats. On 5 of these no effect was seen with the currents used; on the other two cells harmine reduced the depressions by both glycine and GABA in parallel, but only at a stage when the spike height was markedly reduced.

Responses to noradrenaline and 5-hydroxytryptamine

The actions of both NA, (−6 to 100 nA, mean 32 nA) and 5-HT, (8–80 nA, mean 43 nA) were tested fully on 34 unidentified spinal neurones in 7 barbiturate anaesthetized and two decerebrated rats. All these cells responded with a reduction in their DLH-induced firing rates. The latency to the beginning of these depressant responses was 0–10 sec, and the maximum depression would occur with a latency of between 3 and 30 sec.

Primarily excitatory responses were never seen with either drug, but biphasic (depressant–excitant) responses did occur; of the 34 cells, 7 showed late excitatory response to NA, and 18 to 5-HT; 5 of these cells responded in this way to both amines. With 2 of these 5 cells the excitatory responses were considerably greater than the shorter latency depressant responses; more often the excitatory response was similar in size to, or smaller than the early depressant responses. Further, the late excitation to 5-HT was usually greater (higher maximum frequency and increased duration) than was that to NA. An example of a cell showing late excitation to 5-HT but not to NA is shown in Fig. 2C. No difference was seen in the threshold (in terms of ejecting current) of the depressant and excitant responses to the amines; however, this was not investigated systematically.

With most of these 34 cells, we were testing harmine and LSD for any action on the responses to NA and 5-HT; for this reason the amines were ejected from the electrodes in a regular cycle. Consequently the applications of the amines were kept as short as was compatible with reproducible responses. In this situation the late excitatory response of those cells showing biphasic responses occurred after the termination of the current ejecting the amines, as in Fig. 2C. Frequently however,

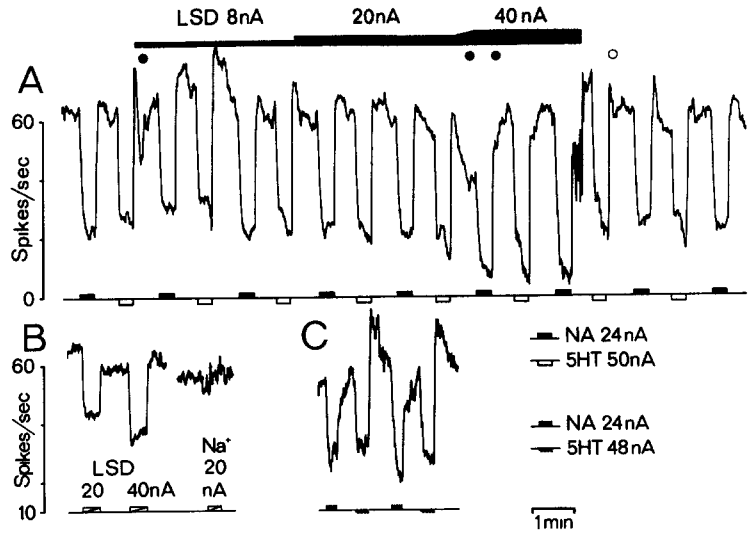

Fig. 2. A: actions of LSD on the responses of a spinal neurone to the alternate application of noradrenaline (NA) 24 nA and 5-hydroxytryptamine (5-HT) 50 nA. The firing rate was maintained by the continous application of DL-homosyteate, the current of which was increased (filled black circles) to compensate for the depressant effect of LSD, and decreased again (open circle) at the end of the LSD application. LSD 8 and 20 nA had no effect on the amine responses. Increasing the current to 40 nA resulted in increased amine responses, but the action potential height was reduced at the same time. Just before the termination of the LSD ejection, the action potential height became more severely reduced and the cell began to discharge in bursts. The effects of LSD reversed within a minute of terminating the ejection. B: same cell as in A, showing the depressant effect of LSD 20 and 40 nA. Sodium ion at 20 nA had no effect. C: different cell which responded to 5-HT, but not to NA, with biphasic inhibitory–excitatory responses. The two cells were from different pentobarbitone anaesthetized preparations.

the firing rate was already increasing at the termination of the ejection, the peak of depression of neuronal firing having occurred before the end of the amine ejecting period. In spite of this, the depression was never seen to reverse into excitation during the application of an amine, even when this application was prolonged up to a minute or so.

Four of the 34 cells tested were examined in the two decerebrate preparations. Of these 4 cells, 3 showed biphasic responses to NA and 2 to 5-HT. The time course of responses was similar to responses obtained in the 7 pentobarbitone-anaesthetized animals.

Some other spinal cord neurones were seen which were apparently unresponsive to NA and/or 5-HT, at least with ejection currents of up to 80 nA or so. No attempt was made to estimate the proportion of unresponsive cells. It is however our impression that relatively very few cells failed to show any response to ejection currents in the range employed.

The effects of harmine on amine responses

Harmine (5–50 nA, mean 26 nA) was tested on the amine responses of 26 of

the 34 cells described above. With 18 of these 26 cells harmine reduced the DLH-induced firing rate, but this effect was often closely followed or was accompanied by changes in action potential configuration. In no case was antagonism of NA or 5-HT depressions seen in the absence of effects on the action potentials.

No antagonism of late excitation was seen with 3 cells tested in the two decerebrate rats, but antagonism of the late excitatory phase of biphasic responses did occur in anaesthetized preparations. In the latter group, harmine was tested on 3 of the cells which showed late excitations to both NA and 5-HT, and reduced the excitations to both amines with 2 of these. It was tested on one cell which showed late excitation to NA only, and reduced this excitation, but only at the same time as causing spike changes. It was also tested on 7 of the cells which showed late excitation to 5-HT only, and reduced the excitation with 4 of these. However, recovery of excitation was never seen after periods of up to 20 min following the termination of harmine ejection. The significance of these observations must therefore remain obscure.

The effects of LSD on neuronal firing rates and on amine responses

The actions of LSD (0–52 nA, mean 21 nA) were investigated on 23 spinal neurones in 9 rats (of which 3 cells were in two decerebrate preparations). In all but 3 cells LSD depressed DLH-maintained neuronal firing rates. However, as with harmine, this effect was frequently followed by changes in spike configuration, and so it was not always possible to determine whether the decreased discharge rate reflected a genuine depression rather than non-specific membrane effects. In some cases however, currents of LSD which began to reduce the DLH-induced firing could be increased fourfold or more before changes in spike configuration became apparent. Further, brief (10–30 sec) pulses of relatively high (40–60 nA) currents of LSD caused clear depressions of discharge rates without concomitant affects on spike configuration, whilst similar currents of Na^+ had little or no effect on the rate (see Fig. 2B).

LSD was tested for any interaction with NA or 5-HT (current ranges as above) on 22 of these 23 spinal neurones. With 3 cells (barbiturate preparations) the depressant responses to both amines were increased by LSD. More commonly the depressant responses to both amines were decreased in parallel, but only at the same time as, or just before, changes in spike configuration became apparent. In one case each, NA and 5-HT depressions appeared to be relatively specifically antagonized.

An example of the effects of LSD on a cell which responded to NA and 5-HT with simple depressions is shown in Fig. 2A and B. In Fig. 2A LSD was applied with increasing currents during the alternate application of NA and 5-HT. LSD itself reduced the DLH-maintained firing rate (see Fig. 2B), and so the current of DLH was increased to compensate for this (filled circles above trace). As soon as the LSD current was increased to 40 nA, the spike configuration was affected; at the same time the responses to NA and 5-HT became greater. Just before the LSD current was terminated, the action potential height became much reduced and the discharge pattern showed bursts of activity. All these effects were reversed a few seconds after terminating the LSD current; the open circle above the trace indicates when the DLH current was reduced to compensate for the increase in firing rate.

As with harmine, more specific effects of LSD were seen on the late excitations caused by NA and 5-HT. LSD was tested on 3 cells (one barbiturate, two decerebrate) showing late excitations to both NA and 5-HT; the excitations of 2 of these were antagonized. No effect was seen on one cell (barbiturate preparation) showing late excitation only to NA. With 7 of 10 cells (barbiturate preparations) showing late excitation to 5-HT alone, the late excitation was reduced or abolished during the ejection of LSD. Unfortunately, however, these excitant responses seldom recovered completely, at least during the time allowed after the termination of the LSD ejection. In all, 9 cells showed antagonism of late 5-HT excitation by LSD. No recovery at all was seen with 3 cells, whilst almost full recovery was observed with 4 cells.

No significant differences were seen between the 3 cells tested in decerebrate animals and the other cells tested in pentobarbitone anaesthetized animals.

DISCUSSION

In common with their effects at other sites, the actions of 5-HT and NA on rat spinal neurones are more varied and irregular than, for example, are those of amino acids. It is possible that the different types of response seen in the present experiments were associated with different classes of neurones, since no attempt was made to identify cells either anatomically or physiologically. It would appear, however, that there are at least two types of receptors available for both monoamines, one mediating depression and the other excitation. The time course of the depressant response was shorter than that of the excitation. Further, it seems that the depressant response was more powerful than the excitant response, since excitation was never seen to overcome the depression until after the end of the application of the amine.

A similar diversity of responses to that described here has been reported in the cat spinal cord[4,15,16,29,31,33]. However, there were marked differences between the responses of parasympathetic and those of sympathetic preganglionic neurones to the ejection of 5-HT[19,31]. It is unlikely that non-specific facilitation following depression of firing[16] could explain the late excitation seen in the rat; with several cells only one of the equi-effective depressant responses to 5-HT and NA was followed by excitation; in addition such long lasting excitations were never seen following the ejection of glycine or GABA.

In other regions of the cat central nervous system up to 4 types of response have been described for each amine (see refs. 9 and 26 for references).

In the rat, a similar diversity of responses has been reported for NA and/or 5-HT in other parts of the nervous system[1,11,12], although only one type of response to 5-HT, namely depression, was seen with identified raphe neurones[1]. Although primary excitatory responses to 5-HT have not been seen in the present study in the rat spinal cord it is unlikely that densensitization[10,23,28,32] prevented such an observation. Probably more pertinent to this finding is the pH of the NA and 5-HT solutions used; values of between 3.8 and 4.2 were used in the present study, levels probably high enough to avoid the excitatory effects of hydrogen ion[17,18,25]. The only other reports so far published on responses in the rat spinal cord suggest that

5-HT facilitates the monosynaptic reflex as well as dorsal and ventral root potentials[2,3].

Barbiturates have been variously reported to reduce excitatory responses to monoamines[11,24,30], increase the excitant effect of 5-HT[19] and not to affect NA excitation[8]. Although it is possible that pentobarbitone may have masked excitatory effects of 5-HT in our experiments on rat spinal neurones, the results from 4 cells in two decerebrate rats do not support such a contention.

In the present experiments, LSD was never observed to affect amine depressions without concomitant or rapidly following effects on action potential configuration. Rather it depressed amino acid-induced neuronal firing rates. However, it did frequently reduce the late excitations to 5-HT, and, with two cells, those to NA. These findings are compatible with numerous reports in the literature which suggest that LSD does not antagonize 5-HT depressant responses, only its excitant effects; only with rabbit olfactory bulb neurones has LSD been reported to antagonize 5-HT depression[7], and then the antagonism was not specific to 5-HT. However, the lack of adequate recovery in our experiments of many of the excitatory responses (see also ref. 10) following the termination of LSD ejection throws doubt on the validity of this finding.

The depression of DLH-induced firing rates is compatible with the reports from workers that systemically administered LSD can mimic 5-HT depression at various sites, but electrophoretic confirmation of such reports is more limited[1,20]; it is however possible that there is some more specific antagonism between LSD and excitant amino acids[10].

The apparent potentiation of 5-HT and NA depressions by LSD with 3 cells appears to be a finding not previously reported. We have no evidence to suggest how this effect may have been mediated.

The only previous reports of the actions of β-carbolines on the responses of single cells to transmitter candidates concerned 5-HT. In these cases, harmine was without effect[27] and harmaline had weak agonistic as well as antagonistic actions[13]. In our experiments harmine did show a tendency to irreversibly reduce the excitant effects of both 5-HT and NA, but its action was inconsistent. Although harmaline showed some specificity as an antagonist both of glycine and of acetylcholine on rat spinal neurones, no such action could be demonstrated for harmine.

These effects of the β-carbolines, as well as an apparently non-specific membrane effect, occurred with doses (coulombs of ejecting current) considerably lower than those generally required to induce rhythmical activity in the olivary nucleus. However the interactions being studied in the spinal cord probably occur on or near the soma of single neurones whereas the site of action of the rhythm-inducing drugs in the olivary nucleus is likely to be more widespread. The action of the β-carbolines underlying spike height reduction of spinal neurones is unlikely to apply to these substances when they are ejected into the inferior olive as at this site the early component of unitary spikes is not significantly reduced even during established rhythmical activity[22].

The present series of experiments thus failed to demonstrate any specific action of the β-carbolines on spinal neurones such as might help to explain their ability to induce rhythmical activity in the inferior olivary nucleus.

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