Br. J. Pharmac. (1976), **57**, 191–199

ANALYSIS OF THE EFFECTS OF *p*-METHOXY-PHENYLETHYLAMINE ON SPINAL CORD NEURONES

L.M. JORDAN & D.A. McCREA

Department of Physiology, University of Manitoba, Faculty of Medicine, Winnipeg, Manitoba R3E 0W3, Canada

1 Para-methoxyphenylethylamine (PMPEA) was applied microiontophoretically onto interneurones and motoneurones in the spinal cords of acute spinal cats anaesthetized with α-chloralose. Its effects were compared with those of noradrenaline (NA) and 5-hydroxytryptamine (5-HT).

2 PMPEA had effects on interneurones which were similar to those of NA and/or 5-HT; its action was predominantly depressant, and it rarely affected interneurones which could not be influenced by NA or 5-HT.

3 The actions of PMPEA on interneurones excited by electrical stimulation of leg nerves showed that the population of interneurones influenced by the drug coincides with the population affected by NA and 5-HT and by intravenously administered PMPEA.

4 Renshaw cells, which are excited by intravenous PMPEA, were either not affected or depressed by microiontophoretically applied PMPEA.

5 Alpha motoneurones, which are depolarized by intravenous PMPEA, were hyperpolarized by microiontophoretically applied PMPEA. Antidromic firing of the cells could be blocked by PMPEA.

6 The differences between the effects of intravenous infusion and the iontophoretic application of PMPEA upon motoneurones is most easily explained by inhibition of interneurones and a concomitant disinhibition of motoneurones. A similar mechanism may also account for the different effects seen with intravenous and iontophoretic application of PMPEA on Renshaw cells.

Introduction

The effects of intravenously administered *p*-methoxyphenylethylamine (PMPEA) on the cat spinal cord have been previously described (Walker, Willis & Willis, 1970; Jordan, Willis & Matthews, 1972; Jordan & Willis, 1973; Willis, Ashkenazi, Willis & Haber, 1973). In doses of 2.5 mg/kg or greater it enhances monosynaptic reflexes of both flexor and extensor motoneurones (Walker *et al.*, 1970), and this reflex enhancement is accompanied by depolarization of the membrane potentials of α-motoneurones and by decreased polysynaptic excitatory and inhibitory polysynaptic potentials recorded from motoneurones (Jordan *et al.*, 1972). It also interferes with transmission in interneuronal pathways in the spinal cord, it depresses the firing of single interneurones in the spinal cord grey matter, and it reduces synaptic noise in motoneurones (Jordan & Willis, 1973).

These actions of intravenously administered PMPEA are due to a central action of the drug on the spinal cord (Walker *et al.*, 1970), and various lines of evidence suggest that these effects on central nervous system neurones are due to an action on monoaminergic synapses. It is a sympathomimetic agent (Epstein, Gunn & Virden, 1932), and its hypertensive action, like that of tyramine, can be reduced by reserpine or guanethidine pretreatment, suggesting that it causes release of noradrenaline (NA) from nerve terminals (Cession-Fossion & Michaux, 1963). Paton & Pasternak (1974) have shown that PMPEA accelerates the efflux of NA from atria, and Horn (1973) showed that it may cause inhibition of catecholamine uptake. PMPEA also blocks the uptake by isolated synaptosomes of NA and 5-hydroxytryptamine (5-HT) (Ashkenazi, Haber, Coulter & Willis, 1973), and it can reduce the levels of both NA and 5-HT in the spinal cord of mice (Ashkenazi, Willis & Haber, 1974). The effects of PMPEA on reflexes can be antagonized by blockers of α-adrenoceptors and tryptamine receptors (Walker *et al.*, 1970), and its actions on monosynaptic reflexes, on excitatory and inhibitory interneuronal pathways to motoneurones, and on the pathways causing primary afferent depolarization are similar to those of monoamine precursors and other substances which increase monoamine levels in the spinal cord (for references, see Jordan *et al.*, 1972 and Jordan & Willis, 1973). The enhancement of reflexes produced by PMPEA is potentiated by the monoamine precursors 5-hydroxytryptophan (5-HTP) and 3,4-dihydroxyphenylalanine (L-DOPA) and by the

monoamine oxidase inhibitor, nialamide (Coulter, Bird, Willis & Willis, 1972; Willis *et al.*, 1973). All of these studies strongly support the notion that PMPEA acts at noradrenergic as well as 5-hydroxytryptaminergic synapses in the spinal cord.

Previous studies conducted with intravenously administered PMPEA have not revealed whether its actions on motoneurones and interneurones are due to direct postsynaptic effects or to release of monoamines from terminals within the spinal cord, nor have they shown whether the effects of PMPEA are limited to monoamine synapses. Furthermore, it is necessary to reconcile the depolarizing effect of intravenously administered PMPEA on motoneurones with the finding that microiontophoretically applied NA and 5-HT cause hyperpolarization of these cells (Phillis, Tebecis & York, 1968; Engberg & Thaller, 1970; Engberg & Marshall, 1971). Accordingly, the effects of microiontophoretically applied PMPEA on spinal cord interneurones, Renshaw cells and motoneurones have been determined and compared with those obtained with NA and 5-HT. A preliminary report of some of these results has been presented (Jordan, 1972).

Methods

Twenty-six cats were used in these experiments. The animals were anaesthetized with α-chloralose (80 mg/kg, i.v.) and the spinal cord was transected at the level of L1. After laminectomy, the L6, L7 and S1 ventral roots were cut, and the central end of L7 or S1 was mounted for stimulation on bipolar platinum electrodes. The leg nerves posterior biceps-semitendinosis (PBST), semimembranosis-anterior biceps (SMAB), gastrocnemius-soleus (GS), tibalis anterior (TA), and sural (S) were cut, and the central ends were mounted on bipolar platinum electrodes for stimulation. Constant current stimuli of 0.1 ms duration were applied with a Grass S88 stimulator and a Grass Constant Current unit. Carotid artery blood pressure was continuously recorded on a penwriter, and rectal temperature was maintained at 37°C using a heating pad and a feed-back circuit.

Seven-barrelled micropipettes with tip diameters of 4–8 μm were used for recording extracellular action potentials and for administering drugs microiontophoretically. The central recording barrel contained 2M NaCl, while the surrounding barrels were filled by centrifugation with various combinations of the following solutions: noradrenaline bitartrate (Sigma; 0.2M, pH 5.0), 5-hydroxytryptamine creatinine sulphate (Sigma; 0.04 M, pH 6.0), *p*-methoxyphenylethylamine hydrochloride (Calbiochem, 0.2 M, pH 4.5), L-glutamate, monosodium salt (Sigma; 0.2 M), acetylcholine chloride (Sigma, 0.2 M), and NaCl (Baker; 2 M). Drugs (except glutamate) were ejected as cations by passing anodal currents from constant current sources (Spencer, 1971) through the microelectrodes. Cations were prevented from diffusing from the drug barrels by applying cathodal retaining currents of 8–10 nA, whereas glutamate was retained by passage of anodal currents and ejected by passing cathodal currents. An effect of a drug on neuronal firing rate was judged to be genuine if recovery occurred, if the effect was repeatable, and if it was not mimicked by a current control. A drug was judged to have no effect if a 1 min application at a current of 150 nA or more failed to produce a change in firing rate or membrane potential. Cationic current controls were performed by passing Na^+ from a barrel filled with NaCl.

When intracellular recording with simultaneous extracellular drug applications was attempted, three-barrelled microelectrodes for extracellular recording and drug applications were positioned in Plexiglass holders similar to that described by Phillis *et al.* (1968). Intracellular records were achieved through the use of an attached fine-tipped microelectrode (filled with 2 M KCl or 3 M NaCl), which fitted into a groove between two adjacent barrels of the multi-barrelled microelectrode and projected 20–60 μm beyond the tips of the drug-containing micropipettes. The arrangement allowed simultaneous drug application and intracellular as well as extracellular recording. The intracellular recording barrels had resistances of 10–20 megohms. Resting potentials were observed with a d.c.-coupled amplifier and were recorded by photographing the oscilloscope screen at 3 s intervals.

Extracellularly recorded potentials were amplified and displayed on an oscilloscope for photography. Effects of drugs on synaptically evoked firing were determined from filmed records. Spontaneous and glutamate-evoked firing rates were recorded through the use of a window discriminator and an electronic counter (Hewlett-Packard 5214L). Only units which could be clearly distinguished from background noise were accepted for analysis. The output of the counter was converted to analogue form and displayed on a pen recorder. Effects of drugs on firing rate were determined from the pen recorder records.

The synaptically-evoked firing of interneurones and motoneurones, as well as antidromic firing produced in motoneurones, was recorded by photographing the oscilloscope screen. Interneurones were identified by their failure to respond to ventral root stimulation and by their characteristic discharge of multiple action potentials in response to a single stimulus applied to a peripheral nerve. Motoneurones and Renshaw cells were identified by their response to ventral root stimulation.

A ball-tipped silver–silver chloride electrode was placed at the dorsal root entry zone and was used to detect the arrival at the spinal cord of afferent volleys evoked by leg nerve stimulation. Interneurones which

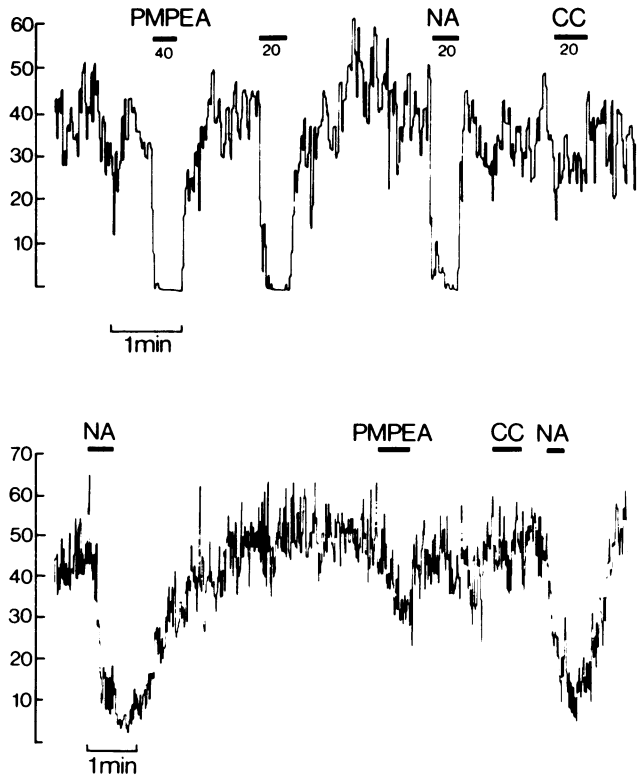

Figure 1 The effects of *p*-methoxyphenylethylamine (PMPEA) and noradrenaline (NA) on spontaneous firing of two spinal interneurones. The ordinates represent firing rate in spikes per second. The firing of the neurone illustrated in the upper trace was depressed by applications of PMPEA of 40 and 20 nanoamperes (nA) during the periods indicated by horizontal bars. The effects of NA (20 nA) and current control (CC) are also illustrated. In the lower trace, the responses to drugs and current control (100 nA in all cases) are illustrated.

fired a spike within 0.5 ms after the arrival of the afferent volley at the surface of the spinal cord were considered to be monosynaptically activated. Stimulus strengths required for activation of low threshold and high threshold afferent fibres in leg nerves were determined from the cord dorsum record; stimulus strengths which produced the first detectable negative waves on the cord dorsum record were considered adequate for exciting low threshold afferent fibres. Effects which could be produced only by stimulus strengths more than ten times threshold for cord dorsum evoked potentials were considered to be due to activity in high threshold afferents.

Results

Effects of microiontophoretically applied p-*methoxyphenylethylamine, noradrenaline and 5-hydroxytryptamine on interneurones*

PMPEA was ejected microiontophoretically in the vicinity of 71 interneurones in the lumbar enlargement. The drug was tested on spontaneous firing of 30 of these; 35 were excited by stimulation of peripheral nerves (on three of the latter PMPEA was also tested on spontaneous firing). Figure 1 illustrates the effects of PMPEA and NA on two different spontaneously firing interneurones. NA had a powerful depressant action on both of these neurones, and PMPEA also depressed them. The reduction in firing rate caused by these drugs was not mimicked by control currents passed from an adjacent barrel containing NaCl. In general, PMPEA either had no effect on cells influenced by NA or 5-HT, or its effects were qualitatively similar to those of the monoamines. PMPEA was nearly always without effect on cells that were not influenced by NA or 5-HT. Table 1 shows the distribution of effects obtained with NA, 5-HT and PMPEA. These findings suggest that the action of PMPEA on spinal interneurones is primarily one of depression, and that its effects on interneurones may be dependent upon the presence of monoamine receptors. Table 2 is a comparison of the effects of PMPEA with those of NA and/or 5-HT on the same

Table 1 Effects of *p*-methoxyphenylethylamine (PMPEA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) on spontaneously firing, glutamate-activated and orthodromically activated spinal cord interneurones

	% Excited	*% Depressed*	*% Unaffected*
Orthodromically activated			
PMPEA (35)*	0	11	89
NA (50)	4	32	64
5-HT (34)	2	19	79
Spontaneously firing†			
PMPEA (30)	3	40	57
NA (41)	12	56	32
5-HT (18)	5	39	56
Glutamate-activated			
PMPEA (9)	0	100	0
NA (19)	0	68	32
5-HT (7)	0	86	14

* Numbers in brackets indicate total number of cells tested with each drug in each category; † 3 cells in this category were also tested using orthodromic activation.

Table 2 Comparison of the effects of *p*-methoxyphenylethylamine (PMPEA) with those of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) on the same cells

PMPEA		*NA*			*5-HT*		
		No. excited	*No. depressed*	*No. unaffected*	*No. excited*	*No. depressed*	*No. unaffected*
No. excited	2	1	1	0	1	0	0
No. depressed	24	0	20	1	0	12	3
No. unaffected	41	4	12	22	1	2	25

Table 3 Effects of *p*-methoxyphenylethylamine (PMPEA) on orthodromically activated spinal cord interneurones

Source of excitation	*PMPEA*		
	No. excited	*No. depressed*	*No. unaffected*
Monosynaptically excited from low threshold muscle afferents (2)*	0	0	2
Monosynaptically excited from high threshold muscle afferents (3)	0	1	2
Polysynaptically excited from high threshold muscle afferents (5)	0	1	4
Polysynaptically excited from skin afferents (10)	0	0	10
Polysynaptically excited from skin afferents and high threshold muscle afferents (15)	0	2	13

* Numbers in brackets indicate the number of neurones in each category.

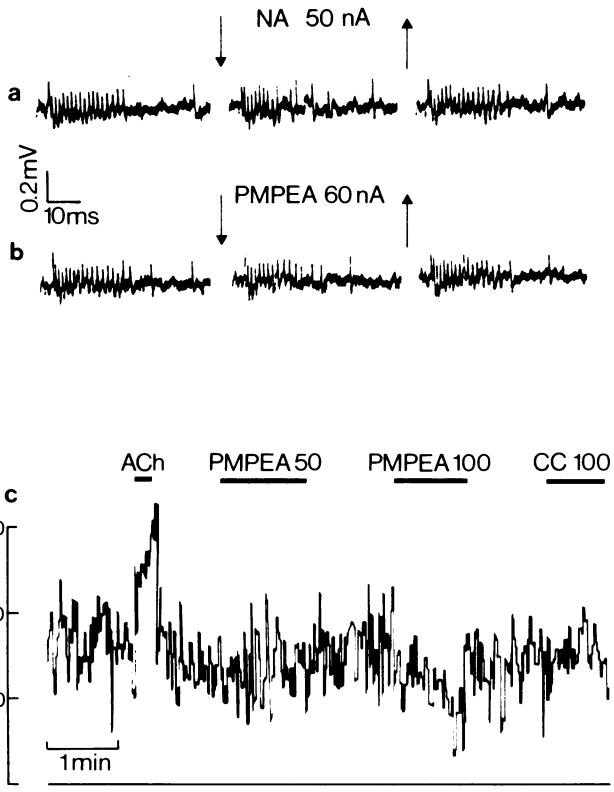

Figure 2 The actions of noradrenaline (NA), acetylcholine (ACh) and *p*-methoxyphenylethylamine (PMPEA) on Renshaw cells: (a) and (b) are photographs of oscilloscope traces of burst discharges produced by ventral root stimulation. Drug applications occurred between the arrows. No effect was observed when a subsequent current control was applied. (c) is a ratemeter record of spontaneous firing of a second neurone. The ordinate represents spikes per second. Drug application occurred during the time period indicated by the horizontal bars. ACh was applied using a current of 15 nA.

interneurones. It can be seen that the neurones which were depressed by PMPEA were also consistently depressed by NA and/or 5-HT. Only 1 of 24 interneurones depressed by PMPEA and also tested with NA or 5-HT was uninfluenced by NA or 5-HT.

In agreement with Engberg & Ryall (1966), it was found that firing of interneurones produced by orthodromic activation of afferents in leg nerves was more difficult to influence by microiontophoretic applications of NA and 5-HT than spontaneous or glutamate-evoked firing (Table 1). The same was true for PMPEA, which depressed only 4 of 35 orthodromically excited interneurones and had no effect on the remainder.

Table 3 summarizes the effects of PMPEA on orthodromically excited interneurones. It can be seen that the interneurones depressed by PMPEA were excited by stimulation of skin and high threshold muscle afferents (flexion reflex afferents). This population of interneurones coincides with that depressed by intravenous infusion of PMPEA (Jordan & Willis, 1973).

The effects of NA, 5-HT and PMPEA were determined on 10 Renshaw cells identified by their response to antidromic stimulation of the ventral root. The effects of NA and PMPEA on synaptically evoked firing and of NA, acetylcholine (ACh) and PMPEA on spontaneous firing of Renshaw cells is illustrated in Figure 2. NA and PMPEA reduced the number of action potentials produced in response to ventral root stimulation (Figure 2a and b). Figure 2c shows the response of a spontaneously firing Renshaw cell to ACh and PMPEA. This cell was markedly excited by ACh, showing that the firing rate was sufficiently low to allow development of an excitatory response. Nevertheless, PMPEA had only a mild depressant action on this cell. PMPEA depressed the spontaneous or synaptically evoked firing of 6 of the

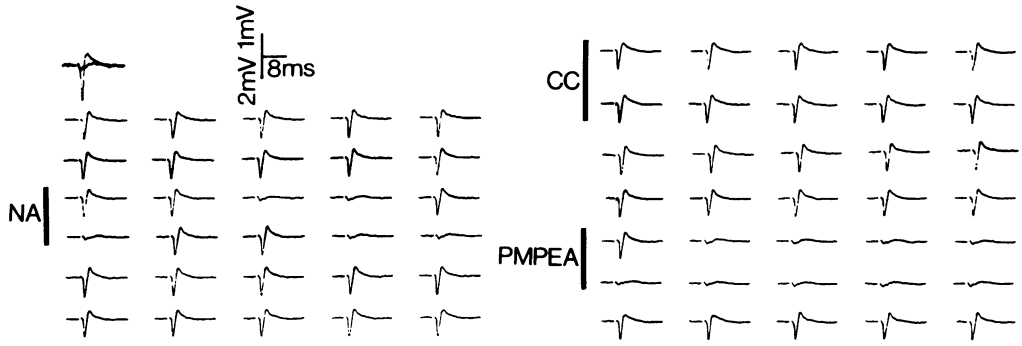

Figure 3 Effects of *p*-methoxyphenylethylamine (PMPEA) and noradrenaline (NA) on antidromic firing of a motoneurone. Stimulation of the L7 ventral root at threshold (2 superimposed traces at upper left) revealed the all-or-nothing nature of the spike (negative downwards). The 1 mV calibration applies to this record. Subsequent traces at lower gain are consecutive records reading from left to right taken at 3 s intervals. Drugs and control current (CC) were applied during the periods indicated by vertical bars. Currents of 100 nA were used in each case. These effects were obtained using a multibarrelled extracellular electrode.

10 Renshaw cells studied and had no effect on the remaining 4. NA depressed 5 of the same 10 cells and had no effect on the remainder. 5-HT was tested on 7 Renshaw cells, having no effect on 5 and a depressant action on 2. ACh always excited the Renshaw cells on which it was tested. The effects obtained with NA and 5-HT are in agreement with the depressant action of NA on these cells reported by Engberg & Ryall (1966). The fact that PMPEA can mimic these effects suggests that the drug may interact with the same receptors as those for NA or 5-HT. The failure of microiontophoretically applied PMPEA to produce excitation, however, is not similar to the effects obtained on recurrent inhibition and Renshaw cell discharge observed when PMPEA is infused intravenously (Jordan & Willis, 1973).

Effects of p-*methoxyphenylethylamine and noradrenaline on α-motoneurones*

Since PMPEA routinely causes depolarization of motoneurones when infused intravenously, it was of interest to determine the direct effect of PMPEA on motoneurones and to compare it with the well documented hyperpolarizing action of NA on these cells (Phillis *et al.*, 1968; Engberg & Thaller, 1970; Engberg & Marshall, 1971). The effects of these drugs on extracellularly recorded firing of motoneurones were determined. Motoneurones were identified by the presence of action potentials with discrete thresholds to antidromic stimulation of ventral roots which followed stimulation at high rates (up to 200/second).

Figure 3 shows the type of response caused by NA on motoneurone firing produced by peripheral nerve or ventral root stimulation. In agreement with Weight & Salmoiraghi (1967), the extracellularly-recorded spikes can be blocked by NA. NA blocked antidromic firing in 5 of 11 motoneurones and had no effect on the remainder. Figure 3 also shows the actions produced by PMPEA on extracellularly recorded motoneurone antidromic action potentials, indicating that microiontophoretic application of PMPEA, unlike intravenous infusions of the drug, causes depression of motoneurone discharges. PMPEA was applied near 14 motoneurones from which extracellular spikes were recorded; in 4 of these neurones the spikes were blocked during the action of PMPEA, whereas application of PMPEA with currents up to 150 nA had no effect on the remaining 10 neurones. PMPEA never caused firing or increased excitability of motoneurones, regardless of ejecting current.

Intracellular records were obtained from over 60 motoneurones with extracellular microiontophoretic application of NA and/or PMPEA. Initial resting potentials ranged from 44 to 72 mV. Three-barrelled drug electrodes usually contained NA, PMPEA and NaCl. The NaCl barrel could be used for applications of control currents as well as for extracellular recording. The proximity of the drug electrode to the impaled motoneurones could be assessed by observing the simultaneously recorded intracellular as well as extracellular actions potentials. The intra- and extracellular electrodes were judged to be recording from the same neurone if the action potentials recorded from both had identical thresholds, latencies, and ability to follow high frequency stimulation. Changes in membrane potential were judged to be genuine effects of the drug if they occurred in cells with stable membrane potentials, if they recovered fully after drug application was terminated, and if the effect was not mimicked by a positive control current applied through the extracellular NaCl barrel.

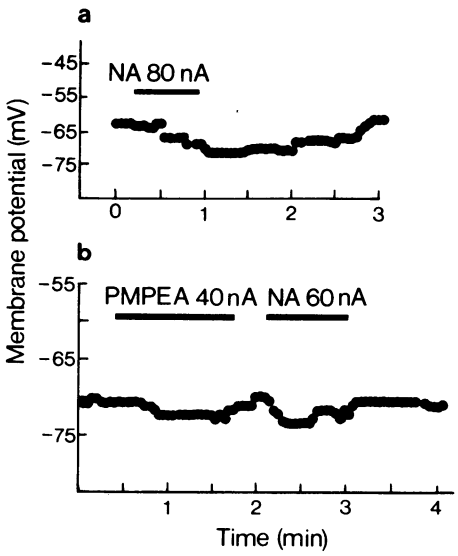

Figure 4 Resting membrane potential changes in motoneurones resulting from noradrenaline (NA) and *p*-methoxyphenylethylamine (PMPEA) application. Each dot represents the value of the membrane potential measured from photographs of oscilloscope traces taken at 3 s intervals. Drug applications are represented by horizontal bars.

Figure 4 shows examples of resting membrane potential changes produced by NA and PMPEA. The hyperpolarization produced by NA is consistent with effects on motoneurone membranes previously reported (Phillis *et al.*, 1968; Engberg & Thaller, 1970; Engberg & Marshall, 1971). The average peak hyperpolarization produced in 5 motoneurones by NA was 4 mV (range 2–9 mV). In three other motoneurones with stable resting potentials NA was without effect when applied at currents up to 150 nA. In the remainder of the motoneurones studied in this way, depolarization of the neurone during drug application occurred which led progressively to loss of the cell, presumably due to damage of the membrane by the electrode. In only one of these cells could even partial recovery be observed.

The effects of PMPEA were observed on 12 motoneurones which had stable resting potentials before and after drug application and in which membrane changes produced by the drug recovered fully after termination of drug application. Hyperpolarization was observed in 3 motoneurones, and one of these effects is shown in Figure 4. NA also hyperpolarized this motoneurone. The average hyperpolarization produced by PMPEA was 8 mV (range 2–16 mV). PMPEA (currents up to 150 nA) had no influence on 9 motoneurones with stable resting potentials. In a number of motoneurones PMPEA application was followed by depolarization of the cell which did not recover. In only one of these was partial recovery observed, possibly due to improvement of the recording conditions. Microionotophoretically applied PMPEA was never observed to cause depolarization of motoneurones which resembled that produced by intravenously infused PMPEA (Jordan *et al.*, 1972), indicating that the latter effect cannot be due to direct depolarization of motoneurones by PMPEA.

Discussion

The results of these experiments reveal the type of direct actions PMPEA exerts on spinal cord neurones of various types, and they allow some conclusions to be drawn about the means whereby intravenous PMPEA influences spinal mechanisms. In general, microiontophoretic application of PMPEA reveals that the drug either has no effect on spinal neurones or it depresses them. The findings that the direct action of PMPEA on spinal interneurones is primarily one of depression, and that it tends to occur only on neurones which can also be depressed by monoamines, suggest that the depression of interneurones produced by PMPEA regardless of the route of administration is dependent upon the presence of monoamine receptors on the affected neurones.

This is consistent with the finding that microiontophoretically applied 3,4-dimethoxyphenylethylamine and PMPEA usually had no effect on caudate neurones which were not influenced by dopamine (Zarzecki, 1974). These results are also consistent with the depression of interneurones, motoneurone synaptic noise and transmission in polysynaptic pathways in the spinal cord observed when PMPEA is infused intravenously (Jordan *et al.*, 1972; Jordan & Willis, 1973). They do not, however, reveal whether the depressions produced by PMPEA are due to direct effects of the drug on interneurone postsynaptic membranes or to release of monoamines from nearby noradrenergic or 5-hydroxytryptaminergic presynaptic terminals.

The interneurone population depressed by microiontophoretically applied PMPEA, so far as it has been identified, coincides well with the population influenced by NA (Engberg & Ryall, 1966) and by intravenous PMPEA (Jordan & Willis, 1973). The depressant effects of NA, 5-HT and PMPEA, when they were observed on identified neurones (orthodromically activated neurones, Table 1), were limited to interneurones activated by flexion reflex afferents. Thus, the same population of interneurones seem to be influenced by intravenous PMPEA as well as by microiontophoretically applied PMPEA and monoamines. This suggests that the mechanisms of

action of microiontophoretically applied and intravenously administered PMPEA on interneurones are identical, and that PMPEA selectively influences monoamine synapses on interneurones.

The response of Renshaw cells to microiontophoretically applied PMPEA also resembled the effects obtained with NA and 5-HT on these cells. This depressant action of PMPEA does not resemble the response to intravenously administered PMPEA, however, which was one of enhancement of the burst discharge in response to antidromic stimulation (Jordan & Willis, 1973). It is likely, therefore, that the effect of intravenously administered PMPEA on Renshaw cells is not due to direct effects on these neurones, but is the result of an action on other cells. This could occur subsequent to increased motoneurone activity resulting from the depolarization in motoneurones caused by intravenous PMPEA (Jordan *et al.*, 1972), or it could arise from removal of inhibitory influences from other interneurones (cf. Wilson, Talbot & Kato, 1964) which may be depressed by NA, 5-HT or PMPEA. Although occasional excitant actions of NA on Renshaw cells have been observed in studies using the method of microiontophoresis (Weight & Salmoiraghi, 1965; Engberg & Ryall, 1966), no such excitant actions were observed in this study. In a subsequent study by Weight & Salmoiraghi (1966), a predominance of depressant actions were observed when NA was tested on Renshaw cell firing.

The experiments on the effects of microiontophoretically applied PMPEA on motoneurone excitability and transmembrane potentials clearly show that the depolarization of motoneurones and the enhancement of monosynaptic reflexes produced by intravenous PMPEA cannot be attributed to a direct postsynaptic depolarizing effect of the drug. As was the case with interneurones, the effects of directly applied PMPEA parallel those of NA. It is likely, therefore, that intravenously administered PMPEA facilities monosynaptic reflex transmission and depolarizes motoneurone membranes through an inhibition of interneurones and a concomitant disinhibition of motoneurones, as suggested previously (Jordan & Willis, 1973). The excitation of motoneurones by intravenous PMPEA through effects on interneurones must be more powerful than any direct depressant action the drug might have on motoneurones. The fact that L-DOPA (Baker & Anderson, 1970) and 5-HTP (Andén, Jukes & Lundberg, 1964) cause excitation of motoneurones, presumably due to widespread release of monoamines in the spinal cord, supports the notion that monoamines must have a more powerful effect on motoneurones by way of actions on interneuronal pathways than through actions at monoamine synapses on motoneurones. It seems likely that microiontophoretically applied PMPEA hyperpolarizes motoneurones by an action similar to the hyperpolarizing actions of microiontophoretically applied NA and 5-HT (Phillis *et al.*, 1968; Engberg & Thaller, 1970; Engberg & Marshall, 1971; 1973). This action of PMPEA might be due to an interaction with monoamine receptors on motoneurones. It is also conceivable that PMPEA might hyperpolarize motoneurones due to an interaction with monoamine terminals on motoneurones. Furthermore, it is possible that PMPEA might hyperpolarize motoneurones due to disfacilitation (cf. Hubbard, Llinas & Quastel, 1969). The drug might reduce excitatory input by an action on nearby interneurones, or by reducing the release of excitatory transmitters from synapses on motoneurones, as suggested by Krnjević (1974) as a possible explanation for the hyperpolarizing action of NA.

In order to establish transmitter roles for monoamines in the spinal cord, it is necessary to show that release of NA and 5-HT from the descending monoaminergic pathways produces the same effects on the neurones of the spinal cord as those produced when exogenous monoamines are applied. Experiments with monoamine precursors have failed to establish this, since the effects of L-DOPA (Andén, Jukes & Lundberg, 1966; Baker & Anderson, 1970) and of 5-HTP (Shibuya & Anderson, 1968) on spinal cord activity are not reduced after chronic transection of the spinal cord. Engberg, Lundberg & Ryall (1968) have shown, however, that the action of reserpine after pretreatment with nialamide (i.e., reduced transmission in flexor reflex afferent pathways) is no longer present in chronic spinal cats. The similarity of the effects of PMPEA on flexor reflex afferent pathways (Jordan *et al.*, 1972; Jordan & Willis, 1973) to those of reserpine suggest that PMPEA may have an action on endogenous monoamine stores. This is also suggested by the finding that PMPEA can accelerate the efflux of NA (Paton & Pasternak, 1974) and cause depletion of both NA and 5-HT (Ashkenazi *et al.*, 1974).

This research was supported by a grant from the Medical Research Council of Canada.

References

ANDÉN, N.-E., JUKES, M.G.M. & LUNDBERG, A. (1964). Spinal reflexes and monoamine liberation. *Nature, Lond.*, **202**, 1222–1223.

ANDÉN, N.-E., JUKES, M.G.M. & LUNDBERG, A. (1966). The effect of DOPA on the spinal cord. 2. A pharmacological analysis. *Acta physiol. scand.*, **67**, 387–397.

ASHKENAZI, R., HABER, B., COULTER, J.D. & WILLIS, W.D. Jr. (1973). Mechanism of action of a sympathomimetic drug, para-methoxyphenylethylamine

(PMPEA) on biogenic amines in the vertebrate CNS. Soc. for Neuroscience, 3rd Annual Meeting, Abstracts p. 262.

ASHKENAZI, R., WILLIS, W.D. & HABER, B. (1974). Effects of para-methoxyphenylethylamine (PMPEA) on biogenic amines in the vertebrate CNS. Amer. Soc. for Neurochemistry, 5th Annual Meeting, Abstract.

BAKER, R.G. & ANDERSON, E.G. (1970). The effects of L-3,4-dihydroxyphenylalanine on spinal reflex activity. *J. Pharmac. exp. Ther.*, **173**, 212–223.

CESSION-FOSSION, A. & MICHAUX, R. (1963). Actions pharmacodynamiques comparées de la tyramine et de son dérivé O-methylé. *C.R. Soc. Biol.*, **157**, 683–685.

COULTER, J.D., BIRD, A.M., WILLIS, J.C. & WILLIS, W.D. (1972). Effects of monoamine precursors on enhancement of the spinal monosynaptic reflex by para-methoxyphenylethylamine (PMPEA). Soc. for Neuroscience, 2nd Annual Meeting, Abstracts p. 202.

ENGBERG, I., LUNDBERG, A. & RYALL, R.W. (1968). The effect of reserpine on transmission in the spinal cord. *Acta physiol. scand.*, **72**, 115–122.

ENGBERG, I, & MARSHALL, K.C. (1974). Mechanism of noradrenaline hyperpolarization in spinal cord motoneurones of the cat. *Acta physiol. scand.*, **83**, 142–144.

ENGBERG, I. & MARSHALL, K.C. (1973). Reversal potential for noradrenaline-induced hyperpolarization of spinal motoneurones of cats. *J. gen. Physiol.*, **61**, 261.

ENGBERG, I. & RYALL, R.W. (1966). The inhibitory action of noradrenaline and other monoamines on spinal neurones. *J. Physiol., Lond.*, **185**, 298–322.

ENGBERG, I. & THALLER, A. (1970). Hyperpolarizing actions of noradrenaline in spinal motoneurones. *Acta physiol. scand.*, **80**, 34–35A.

EPSTEIN, D., GUNN, J.A. & VIRDEN, C.J. (1932). The action of some amines related to adrenaline. I. Methoxyphenyl-ethylamines. *J. Physiol., Lond.*, **76**, 224–246.

HORN, A.S. (1973). Structure-activity relations for the inhibition of catecholamine uptake into synaptosomes from noradrenaline and dopaminergic neurones in rat brain homogenates. *Br. J. Pharmac.*, **47**, 332–338.

HUBBARD, J.I., LLINAS, R. & QUASTEL, D.M.J. (1969). *Electrophysiological Analysis of Synaptic Transmission*, pp. 248–253. London: Edward Arnold Ltd.

JORDAN, L.M. (1972). Effect of microelectrophoretically applied para-methoxyphenylethylamine on cat spinal cord neurons. Soc. for Neuroscience, 2nd Annual Meeting, Abstract, p. 209.

JORDAN, L.M. & WILLIS, W.D. (1973). Effect of para-methoxyphenylethylamine on polysynaptic pathways and on interneurones in the cat lumbar spinal cord. *J. Pharmac. exp. Ther.*, **185**, 575–582.

JORDA, L.M., WILLIS, W.D. & MATTHEWS, M.A. (1972). Effects of para-methoxyphenylethylamine on reflexes and motoneurones in the cat lumbar spinal cord. *J. Pharmac. exp. Ther.*, **181**, 53–64.

KRNJEVIĆ, K. (1974). Chemical nature of synaptic transmission. *Physiol. Rev.*, **54**, 418–540.

PATON, D.M. & PASTERNAK, N.L. (1974). Effect of *O*-methylation on the acceleration of efflux of noradrenaline produced by phenethylamine derivatives. *J. Pharm. Pharmac.*, **24**, 273–274.

PHILLIS, J.W., TEBECIS, A.K. & YORK, D.H. (1968). Depression of spinal motoneurones by noradrenaline, 5-hydroxytryptamine and histamine. *Eur. J. Pharmac.*, **4**, 471–475.

SHIBUYA, T. & ANDERSON, E.G. (1968). The influence of chronic cord transection on the effects of 5-hydroxy-tryptophan, *l*-tryptophan and pargyline on spinal neuronal activity. *J. Pharmac. exp. Ther.*, **164**, 185–190.

SPENCER, H.J. (1971). Programmable nanoampere constant current sources for iontophoresis. *Med. Biol. Engng.*, **9**, 693–702.

WALKER, R.J., WILLIS, J.C. & WILLIS, W.D. (1970). The action of para-methoxyphenylethylamine (PMPEA) on monosynaptic transmission in the cat. *Br. J. Pharmac.*, **38**, 106–116.

WEIGHT, F.F. & SALMOIRAGHI, G.C. (1965). Responses of single spinal cord neurons to ACh, NE and 5-HT administered by microelectrophoresis. *Pharmacologist*, **7**, 214.

WEIGHT, F.F. & SALMOIRAGHI, G.C. (1966). Adrenergic responses of Renshaw cells. *J. Pharmac. exp. Ther.*, **154**, 391–397.

WEIGHT, F.F. & SALMOIRAGHI, G.C. (1967). Motoneurone depression by norepinephrine. *Nature, Lond.*, **213**, 1229–1230.

WILLIS, W.D., ASHKENAZI, R., WILLIS, J.C. & HABER, B. (1973). The role of monoamine oxidase in terminating the reflex effects of para-methoxyphenylethylamine (PMPEA). *Tex. Rep. Biol. Med.*, **31**, 423–429.

WILSON, V.J., TALBOT, W.H. & KATO, M. (1964). Inhibitory convergence upon Renshaw cells. *J. Neurophysiol.*, **27**, 1063–1079.

ZARZECKI, P. (1974). The action of *O*-methylated phenyl-ethylamine derivatives on single neurones in the caudate nucleus of the cat. *Fedn. Proc.*, **33**, 245.

(Received August 25, 1975. Revised February 11, 1976)