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Some characteristics of the long-latency component of the evoked muscle response induced by administration of catechol to the anaesthetized rat: a neurophysiological and neuropharmacological investigation

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1 Administration of catechol to rats anaesthetized with urethane produces a central excitatory state during which an EMG consisting of three temporally distinct components (M1, M2 and M3) can be recorded from forelimb and hindlimb muscles to electrical stimulation of cutaneous afferents.

2 The probability of occurrence of all three components was measured in flexor and extensor muscles of fore- and hindlimb and showed that the long latency component (M3) occurred less frequently in hindlimb muscles than forelimb and that its probability of occurrence in hindlimb extensors was significantly reduced as compared to flexors. A possible reflex pathway for this long latency component of the EMG is suggested.

3 Phenobarbitone (5 mg kg^{-1} i.v.) had no significant effect on the probability of occurrence of M1, M2 or M3.

4 These results suggest that the long latency component (M3) is not due to activation of a spino-bulbo-spinal reflex as has been previously suggested.

5 Cholinoceptor blocking drugs were found to reduce significantly the probability of occurrence of M2 and M3 and anticholinesterases to increase the probability of M2. None of the drugs was found to affect the short latency M1 component of the EMG. These results are discussed in relation to the possible reflex pathways of all three components of the EMG.

Introduction

Systemic administration of catechol (1,2 dihydroxybenzene) to anaesthetized rats produces a central excitatory state during which the animal convulses spontaneously and electromyographic activity can be evoked by tactile, auditory (Dewhurst, unpublished observation) or electrical stimulation. The wide spectrum of increased motor activity seen after administration of catechol, i.e. spontaneous and evoked convulsions and tremor, may make catechol a potentially useful chemical model for the study of epileptic seizures, particularly as it is rapidly absorbed and excreted.

This evoked EMG is totally absent in the anaesthetized, non-catechol treated animal and characteristically consists of three temporally distinct components (Figure 1). The reflex pathways giving rise to the two early components (M1 and M2) have been described previously (Angel & Lemon, 1973a, b; Angel & Dewhurst, 1978). It has been suggested that the third, long-latency component (M3) is a spinobulbo-spinal reflex (Angel & Lemon, 1973 a, b; 1975). This component has some characteristics in common with spinobulbospinal (SBS) reflexes since both are abolished by transection of the spinal cord, unaffected by decerebration and they have similar latencies (Lemon, 1971; Shimamura, 1973).

SBS reflexes have been reported in several species (Shimamura, 1973) and have been shown to be excitatory only in flexor motoneurones and also to be abolished by barbiturates. One of the aims of this study was to investigate the evoked muscle response in both flexor and extensor muscles of fore- and hindlimb, and also to examine the effects of phenobarbitone.

Previous work (Angel *et al.*, 1977) has shown spontaneous catechol convulsions to be diminished

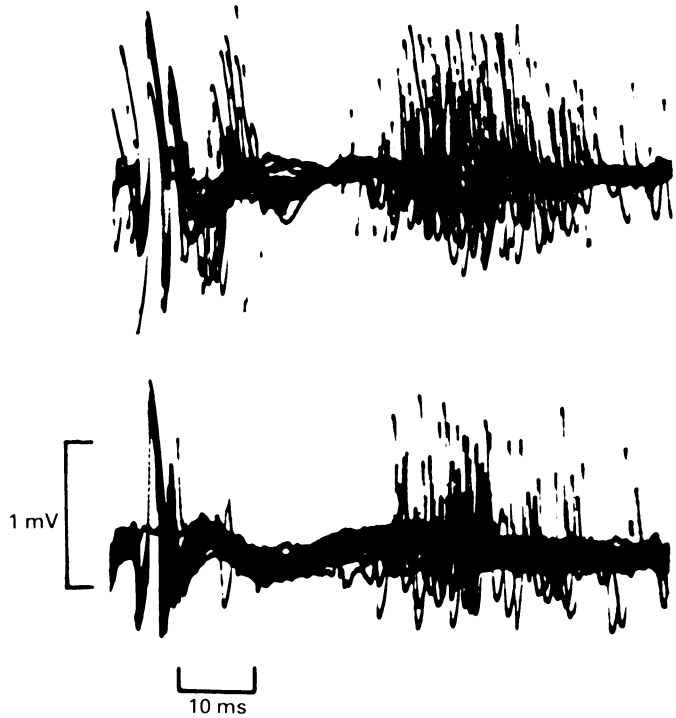

Figure 1 The evoked, electomyogram recorded from flexor carpii to electrical stimulation at the wrist of anaesthetized rats infused with catechol. The upper trace shows a record taken during the control period, the lower one taken 15 min after administration of atropine (1 mg kg^{-1} i.v.). Each record consists of 20 superimposed responses.

by centrally-acting cholinoceptor-blocking drugs and increased in intensity and duration by anticholinesterases. The effect of a number of drugs which modify cholinergic and adrenergic transmission, on the frequency of occurrence of the two early components of the evoked muscle response has also been reported (Angel & Dewhurst, 1978). These results showed that whilst the M1 component was unaffected by any of the drugs used, the probability of occurrence of the M2 component was significantly decreased by cholinoceptor-blocking drugs, indicating that catechol may act, at least in part by increasing transmission at cholinergic synapses within the central nervous system. However, the effects of these drugs on the long-latency component was not measured and this present investigation therefore re-examines more closely the effects of drugs which modity cholinergic transmission on all three components of the electrically evoked muscle response.

Methods

Female albino rats (Sheffield strain) in the weight range 190–210 g were anaesthetized with urethane (1.2–1.4 g kg^{-1} i.p.). Tracheal and jugular vein cannulae were inserted and catechol (25 mg ml^{-1}) dissolved in 0.9% saline was infused intravenously at a rate of 0.02 ml min^{-1} giving a dose of 2.5 mg kg^{-1} body weight min^{-1}. Electromyographic records were taken from flexor and extensor muscles of both fore- (flexor carpi; triceps brachii) and hind-limbs (tibialis anterior; gastrocneumius) using two fine hypodermic syringe needles of fixed separation (1 mm) inserted into the muscle under study. Movement of the limb under investigation was minimized during recording by inserting pins through skin folds of the outstretched limb and the recording electrodes were also rigidly clamped. Recordings were amplified and monitored on a cathode ray oscilloscope and also recorded on magnetic tape.

Muscle responses were evoked by electrical stimulation of the ipsilateral paw applied by means of a strip of gauze soaked in 3 M NaCl tied around the wrist and a steel pin inserted into a digit. This means of stimulation has been shown to excite mainly cutaneous afferents (Angel & Brown, 1967). Stimuli were of 20–50 μs duration, 10–20 V amplitude and applied at a rate of 1/6 s. The stimulus strength was adjusted such that it was just suprathreshold for the

shortest latency component of the response, ensuring that this component had a probability of occurrence of one, during the control period.

The probability of occurrence of all three components of the evoked response was measured as a percentage of the number of stimuli applied. At least 10 min continuous recording was obtained from each muscle of the same animal and this period divided into 2 min sections during each of which 20 stimui were applied. The number of times each component occurred within each section was thus recorded, such that if one component occurred 20 times within one section its probability of occurrence was unity. The latency of all three components was measured directly from the oscilloscope.

Pharmacological experiments

(i) *Effects of drugs that modify cholinergic transmission* Rats were prepared as before. All recordings were made from left forelimb flexor carpi and electrical stimuli were applied to the left forepaw. Drugs were dissolved in saline and administered intraperitoneally in a volume of 0.5 ml. A pre-drug control period was recorded (10–12 min), the test drug administered and recording continued after a suitable period such that the expected peak effects of the drug occurred in the middle of the further 30–35 min recording period. The probability of occurrence of all three components of the evoked muscle response was measured and results were quantified by plotting the probability of occurrence against time after administration of the test drug and subtracting from the area under this curve, the area under the control curve obtained by extrapolating the mean probability of occurrence during the control period. Results were then expressed as percentage difference in areas

$$\frac{(\text{Test}-\text{control}) \times 100}{\text{control}} \text{ in arbitrary units.}$$

In all experiments body temperature was maintained at 37°C ± 1°C by means of an overhead heating lamp and continuously monitored by means of a rectal thermometer.

(ii) *Effects of barbiturate*

The effects of phenobarbitone (5 mg kg^{-1}, i.v.) were also investigated on the probability of occurrence of all three components of the evoked muscle response induced by administration of catechol in albino rats anaesthetized with urethane. Records were taken from left forelimb flexor carpii and the effects of phenobarbitone on M1, M2 and M3 assessed as previously described.

Results

The probability of occurrence of the three components of the evoked muscle response and their respective mean latencies are shown in Table 1 for extensor and flexor muscles of hind- and forelimb. Analysis of the results using a paired *t* test showed no significant difference between discharge probability in flexors and extensors of the same limb for all three components in the forelimb and only for M3 in the hindlimb ($P<0.005$).

The latency results showed no significant difference between flexor and extensor muscles in the same limb for all three components. The latencies for M1 and M2 were significantly longer in hindlimb muscles than forelimb ($P<0.001$) but for M3 there was no significant difference. Although the latency of this component is extremely variable (s.d. = 5 ms), a study of the distribution of latencies did show the majority of forelimb responses to occur in the range 36–40 ms (median 38.4 ms) and the majority of hindlimb responses to occur in the range 41–45 ms (median 41.2 ms).

Table 1 Summary of the probability of occurrence of all three components (M1, M2 and M3) of the evoked electromyographic response induced by administration of catechol, in flexor and extensor muscles of fore- and hindlimb

		Forelimb		*Hindlimb*	
		Flexor	*Extensor*	*Flexor*	*Extensor*
Mean probability of	M1	1.0	1.0	1.0	1.0
occurrence ± s.d.	M2	0.51 ± 0.19	0.48 ± 0.13	0.59 ± 0.18	0.47 ± 0.14
$n = 10$	M3	0.70 ± 0.12	0.65 ± 0.23	0.49 ± 0.18	0.23 ± 0.11
Mean latency (ms)	M1	4.21 ± 0.20	4.26 ± 0.19	8.20 ± 0.47	8.4 ± 0.52
± s.d.	M2	12.20 ± 0.83	12.6 ± 0.96	18.1 ± 1.64	17.6 ± 1.80
$n = 10$	M3	40.0 ± 5.0	39.0 ± 5.0	42.0 ± 4.0	42.0 ± 5.7

Mean latencies of each component are also shown.
n = number of animals

Table 2 The effects of various drugs on the % probability of occurrence of the M2 and M3 components of the electrically evoked muscle response induced by administration of catechol (see methods for explanation)

Drug	*Dose* (mg kg^{-1} i.p.)	*Mean % Difference* $\frac{Test-control}{Control} \times 100$ (Arbitrary units ± s.d.) *M2 Component*	*M3 Component*	n
Atropine sulphate	40	−28.6 ± 17.1*	−23.1 ± 10.7*	7
	1 (i.v.)	−33.0 ± 9.11*	−27.8 ± 8.8*	6
Hyoscine HBr	40	−7.7 ± 17.0	−9.6 ± 6.6	5
Atropine $MeNO_3$	10	+19.9 ± 29.6	+7.03 ± 5.7	5
Mecamylamine	5	−12.2 ± 4.2*	−19.7 ± 5.9*	5
Hexamethonium Br	10	−5.3 ± 14.8	−23.0 ± 15.0	5
Physostigmine	0.03	+11.6 ± 9.7*	−11.8 ± 12.1	7
Neostigmine	0.025	−4.6 ± 10.9	−13.8 ± 8.8	4
Phenobarbitone	5 (i.v.)	−2.5 ± 21.8	−9.8 ± 3.7	5

* Significantly different from paired *t* test at 2% level.
n = number of animals.

Effects of drugs which modify cholinergic transmission

The effects of certain drugs on the probability of occurrence of all three components of the evoked muscle response are shown in Table 2. Vehicle (saline) administration alone did not modify the probability of occurrence of M1, M2 or M3. None of the drugs used had any effect on the probability of occurrence of M1. Atropine and mecamylamine significantly decreased the probability of occurrence of M2 and M3, whilst physostigmine significantly increased that of M2.

Quantitative measurements of the latency of each component were also made by averaging responses (Neurolog Averager NL750) before and after administration of atropine, mecamylamine and physostigmine. Averages were taken of successive time periods, each containing 128 responses. None of the drugs had any significant effect on the latency of M1, M2 or M3.

Barbiturate

Phenobarbitone (5 mg kg^{-1} i.v.) had no significant effect on the frequency of occurrence of M1, M2 or M3 (see Table 2).

Discussion

Electrical stimulation of cutaneous afferents evokes a response in muscles of the same limb in anaesthetized rats, after administration of catechol. This response consists of three components of clearly defined latencies, each of which is dependent upon activation by catechol of a different reflex pathway. The short latency (M1) component depends upon a propriospinal reflex, the second (M2) component results from activation of a reflex involving the somatosensory cortex, whilst the third long-latency component (M3) is of unknown origin (Angel & Lemon, 1973a,b). This third component shares some characteristics with SBS reflexes, in that they have similar latencies, are abolished by transection of the cervical cord and unaffected by decerebration (Lemon, 1971; Shimamura, 1973).

SBS reflexes involve ascending pathways relaying in the bulbar reticular formation and descending the spinal cord to evoke ventral root discharges along all spinal segments bilaterally (Shimamura & Livingstone, 1963). These reflexes have been shown to be excitatory only on flexor motorneurones in many species, and to be inhibitory on extensors (Nagai *et al.*, 1966; Shimamura *et al.*, 1967; Shimamura, 1973). The reflexes have also been shown to be abolished by administration of pentobarbitone and highly sensitive to lack of oxygen (Shimamura, 1973). If the M3 component of the catechol-induced evoked muscle response were dependent upon a SBS reflex, then it would be expected to be absent in extensor muscles and abolished by administration of barbiturates.

The results presented here show clearly that M3 can be evoked in extensor muscles of fore and hindlimb, although relative to flexor muscles of the same limb, it occurs less often in hindlimb extensors. In addition, systemic administration of phenobarbitone (5 mg kg^{-1}) did not significantly reduce the probability of occurrence of M1, M2 or M3 ($P > 0.02$). These results indicate that the M3 component is not dependent upon activation of a spino-bulbo-spinal reflex.

The M3 component is of very variable latency and there is no significant difference between the mean latency of this component recorded from forelimb muscles and that from hindlimb muscles, both being approx. 40 ms. In contrast, the latencies of M1 and M2 components are significantly shorter in forelimb than hindlimb muscles. A comparison of median latencies shows the M3 component recorded in hindlimb muscles to be 3 ms later than that recorded in forelimb muscles and indicate that the reflex pathways involved are fast-conducting. There is some evidence that the sensory pathway may be the dorsal column medial lemniscal system, as for the M2 component since destruction of the dorsal columns at the medullary level has been shown to reduce significantly the frequency of occurrence of M3 (Angel *et al.*, 1976). The relay centre for this reflex would appear to be the cerebellum or cerebellar reticular formation, since it was absent in the decerebellate animal (Lemon, 1971) and it may involve spino-cerebellar sensory pathways (dorsal spinocerebellar tract for hindlimb and cuneocerebellar tract for forelimb) and descending vestibulospinal and reticulospinal pathways. The lower frequency of occurrence of M3 in hindlimb muscles may reflect a less pronounced facilitatory effect of catechol on the different sensory pathways. The large variation in latency of M3 may be the result of activation of different descending pathways.

The probability of discharge of the M2 and M3 components is shown to be significantly reduced by cholinoceptor blocking agents (atropine and mecamylamine) and that of M2 to be increased by physostigmine indicating that catechol may exert its effects by increasing cholinergic transmission at certain central synapses. M1 is not affected by drugs that modify cholinergic transmission. The results for M1 and M2 confirm a previous report (Angel & Dewhurst, 1978). The lack of effect of physostigmine on the probability of discharge of M3 may reflect previous observations of a decreased probability of occurrence of M3 if that of M2 is increased (Dewhurst, 1976) since all three components can be recorded from the same motoneurone (Angel & Lemon, 1973b).

Centrally-acting drugs were found to be far more effective than drugs with similar action which do not cross the blood-brain barrier (compare atropine: atropine methyl nitrate; mecamylamine: hexamethonium; physostigmine: neostigmine) suggesting that catechol exerts its primary effects at central sites. Catechol is known to increase acetylcholine release at the neuromuscular junction (Gallagher & Blaber, 1973) and it is probable that it has a similar action at central cholinergic synapses. Since atropine and mecamylamine were equally effective in reducing the probability of occurrence of M2 and M3, both nicotinic and muscarinic synapses must be involved. The probable site of action of catechol on the cortical reflex pathway, giving rise to M2 has been described previously (Angel & Dewhurst, 1978). If the M3 component is dependent on a cerebellar reflex, then an action on cholinergic synapses in this area would be expected. Acetylcholine functions as an excitatory transmitter in both cerebellar cortex and deep cerebellar nuclei (Phillis, 1971) and both nicotinic and muscarinic receptors have been identified.

Proconvulsants which affect cholinergic mechanisms are not unknown. Several convulsants such as metrazol, bicuculline, picrotoxin and strychnine, although not exerting their major effects on cholinergic systems, do increase the efflux of acetylcholine from the cortical surface, an action associated with their stimulatory effects on underlying cholinergic neurones (Hemsworth & Neal, 1968; Gardner & Webster, 1977). Metrazol has been shown to have stimulatory effects at central muscarinic synapses (Rastogi *et al.*, 1979) and acetylcholinesterase inhibitors such as soman and physostigmine also cause convulsions (Woodbury, 1980; Lundy & Shaw, 1983).

It would thus seem that at least part of the facilitatory action of catechol can be explained by an effect on central cholinergic pathways. The effects on both M2 and M3 indicate an action on different reflex pathways and it may be that catechol is facilitatory at all cholinergic synapses. However, the lack of effect of drugs which modify cholinergic transmission on the spinal reflex pathway (M1) and the fact that even large doses of atropine and mecamylamine administered simultaneously (Dewhurst unpublished observations) do not totally abolish spontaneous catechol convulsions indicates that catechol may also exert some and possibly its major effects on non-cholinergic synapses in the central nervous system.

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