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Factors which may Modify the Pharmacological Action of Curare

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Neuromuscular junctions are remarkably sensitive to pharmacologically active substances. As a result of this, pharmacological analyses have led to the discovery of various important events taking place during transmission.

In 1941, Eccles et al. demonstrated that, in the presence of progressively increasing concentrations of tubocurarine, a gradual depression of the end-plate potential occurs. Their results also showed that when the amplitude of the end-plate potential was below the threshold of the membrane, neuromuscular block set in. Subsequent investigations confirmed these findings and, at present, it is almost generally accepted that tubocurarine combines reversibly with the normal points of attachment (the receptors) of acetylcholine (ACh) and in so doing prevents the action of the transmitter.

Jenkinson (1960) made a quantitative study of the antagonism between ACh and tubocurarine by taking depolarizations of the end-plate region of skeletal muscle as a measure of drug action. The results were consistent with the hypothesis that ACh and tubocurarine compete on a one-to-one basis for the receptors at the end-plate.

More recently Katz and Miledi (1965) confirmed once more the powerful postsynaptic blocking action of tubocurarine and its comparatively small or absent presynaptic effect. Using external micro-electrodes to stimulate non-myelinated motor nerve terminals and to record pre- and post-synaptic responses at the neuromuscular junction of the frog, they demonstrated that tubocurarine caused the end-plate potentials to vanish without interfering with the excitation of the nerve terminal. When repetitive shocks were applied to the terminal, the antidromic impulse was found to follow stimulation at a frequency as high as 200/sec continued for many seconds, even after a dose of tubocurarine chloride of 5×10^{-5} g/ml had been given. In a few experiments, however, there was a slowly developing reduction in the spike amplitude. According to Katz and Miledi it is difficult to know to what extent this effect depended on local damage and progressive deterioration. Possibly in a sufficiently large dose tubocurarine can produce an additive effect.

Because of its mode of action, it is usually said that tubocurarine "raises the threshold of the motor end-plate to ACh" or that it produces a neuromuscular block by "competition with ACh".

The ability of a given dose of tubocurarine to produce a large or a small effect depends on the ease with which the drug can compete with ACh. Thus, various factors such as species, age, sex and so on may undoubtedly increase or decrease the effectiveness of the drug. The duration of the blockade, however, is governed by factors such as the rate of excretion and destruction of tubocurarine, factors which determine how long and in what concentration this drug is present at the neuromuscular junction.

Age

Following STEAD's first communication in 1955 that the response of young children to neuromuscular blocking drugs is different from that of the adults, several workers, amongst whom CHURCHILL-DAVIDSON and WISE (1963) and BACHMAN et al. (1964) reported that in the premature and the new-born infant the neuromuscular junction is resistant to depolarizing drugs and more sensitive to curare. CHURCHILL-DAVIDSON and WISE (1963) also reported that at this early stage a tetanus is poorly maintained and post-tetanic potentiation is not always present. In addition, the blockade produced by depolarizing drugs has some of the characteristics found in patients with myasthenia gravis. For example, tetanic stimulation is poorly maintained and both tetanus and anticholinesterase drugs produce a degree of recovery.

LIM et al. (1964) investigated 134 normal infants and children, varying from 5 weeks to 16 years in age and showed that the variation in the amount of respiratory depression caused by tubocurarine at different ages depends also upon the general anaesthetic agent used. The children were intubated and were breathing spontaneously, having been subjected to minor surgical procedures before investigation. Anaesthesia was maintained post-operatively at plane 1, stage 3 (Guedel), with nitrous oxide and oxygen and either halothane, methoxyflurane or ether. The results showed that under anaesthesia, the younger the children the more sensitive they were to the effects of tubocurarine when the dose is assessed on a body weight basis and that the effects of tubocurarine were enhanced in ascending order by halothane, methoxyflurane and ether. Finally, the results of LIM et al. suggest that the anaesthetic drugs themselves have an age dependent effect upon the neuromuscular transmission. Thus the complementary relaxation provided by some anaesthetics is variable and important.

Animals also show differences according to age. For example, MACLAGAN and VRBOVA (1966) found that the muscles of a 7-day-old kitten are about ten times less sensitive to depolarizing drugs than those of a normal adult cat but very sensitive to tubocurarine.

COUTEAUX in 1941 had already demonstrated that in kittens at birth the nerves end at the surface of the muscle and that the end-plate region

has not yet differentiated, and ORKAND (1964), with the help of the electron microscope, found that even a few weeks after birth only a few synaptic clefts can be demonstrated. Moreover, DIAMOND and MILEDI (1962) reported that the sensitivity of foetal and new-born rat muscle to ACh differs from that of the adult animal. For example, in 19-day foetuses the muscle fibres were quite sensitive to ACh over their entire surface and the same was true of some fibres in animals 1–2 days after birth. Furthermore, spontaneous subthreshold potentials, resembling in most respects the miniature end-plate potentials of adult fibres, could be recorded anywhere. However, the maximal sensitivity observed in foetal fibres was smaller than that of the most sensitive spots in the end-plates of the adult cat and the values tended to be higher with increasing time after birth. Therefore, DIAMOND and MILEDI concluded that embryonic muscle fibres have a general sensitivity to ACh before they receive their motor nerves and suggested that the effect of innervation is to produce a drastic reduction of sensitivity outside the neuromuscular junction.

There are species differences, however. DRACHMAN (1963) made quantitative determinations of the sensitivity of the chick embryo to tubocurarine, suxamethonium and decamethonium at successive stages of development. He used a sufficiently wide range of ages to include the period during which the most striking alterations in end-plate structure could be demonstrated with cholinesterase staining. His results showed that the response of the receptor substance to the neuromuscular blocking drugs is not altered qualitatively or quantitatively during periods of profound change in the localisation of end-plate cholinesterase.

Sex

GALLAGHER and KOCH (1962) showed that female rats were more sensitive to curare than male rats of the same strain and age. The LD50 of tubocurarine administered intraperitoneally was 0.256 mg/kg for female rats and 0.328 mg/kg for male rats. Later on, WOLF et al. (1964) demonstrated that it is between the ages of 1 and 3 months that the female rat develops a marked increase in sensitivity to curare. Gonadectomy, performed when the animals were one week old, failed to abolish the sex difference and the authors concluded that this difference is not only determined by the sex hormones but also by some other sex-linked genetic mechanism.

Denervation

It is well known that the sensitivity of a muscle to depolarizing drugs becomes greater on denervation. JENKINSON (1960) made a quantitative study by taking the depolarization of the muscle fibres as a measure of drug action and showed that the affinity for tubocurarine increases on denervation but not to the same extent as the sensitivity to ACh.

Disuse atrophy and hypertrophy

One of the factors which maintain muscle in a normal functional state is the constraint and tension to which it is subjected by its attachments and by exercise (YOUNG 1946). Together with JEWELL (JEWELL and ZAIMIS 1953), experiments were performed in which this factor has been modified. In cats the tibialis and soleus muscle were tenotomized in order to cause a disuse atrophy and muscles synergistic with soleus were cut to cause soleus to hypertrophy.

The relative insensitivity of the atrophied muscles to tubocurarine was a feature of all these experiments. About twice the dose of tubocurarine necessary to block a normal muscle was required to produce a comparable block in the atrophied muscle. On the other hand, the responses of the hypertrophied muscle to tubocurarine differed in no way from normal. Disuse appears to lower the threshold of the fibre membrane to ACh thus rendering the manifestation of a competitive action more difficult. The suggestion that such a change in sensitivity to acetylcholine occurs in tenotomized muscles would concur with the observations of SOLANDT and MAGLADERY (1942). These authors showed that in the rat disuse atrophy, produced by upper motor neurone lesion, increased the sensitivity of the limb muscles to ACh.

Temperature

Changes in muscle temperature affect the action of neuromuscular blocking drugs. For example, in both animals and man, lowered muscle temperature increases the magnitude of the effect of depolarizing drugs and markedly prolongs their duration of action. In contrast, the magnitude of a block produced by tubocurarine is reduced (ZAIMIS 1956; BIGLAND et al. 1958; CANNARD and ZAIMIS 1959). On rewarming the muscles, these effects are reversed. Moreover, the nature of the blockade appears in no way to be affected however long the paralysis lasts; for example, the action of tubocurarine and that of depolarizing drugs remains antagonistic while neostigmine and edrophonium potentiate the action of depolarizing drugs in both cold and warm muscles.

That tubocurarine is less effective at lowered muscle temperatures was first demonstrated on the isolated diaphragm of the rat by HOLMES et al. (1951). In vivo the analysis of the results obtained is rather more difficult because tubocurarine is slowly destroyed and excreted (MARSH 1952) and successive doses produce cumulative effects.

However, with doses producing less than a 40% reduction of the maximal twitch tension, the effect is always smaller at the lower temperatures. The only experiments in which the blockade produced by tubocurarine is prolonged at lowered muscle temperatures are those in which successive large doses of the drug are administered. It seems that the temperature effects are here masked by the cumulative effects of the drug.

Our experimental results with both depolarizing and curare-like drugs suggested that cooling influences the process by which a long-lasting depolarization of the motor end-plate interrupts neuromuscular transmission. Such a suggestion is supported by the findings of Csapo and Wilkie (1956) who, studying the effect of potassium on frog's skeletal muscles, found that at low temperatures the recovery from the depolarization produced by potassium is very slow and that a brief period of rewarming leads to a sudden and dramatic recovery. A lowering of muscle temperature will undoubtedly have the same influence on the action of acetylcholine as on that of depolarizing drugs. Thus, any increase in the effectiveness of acetylcholine by cooling will automatically reduce the action of any drug trying to compete with it.

Conclusions

A lot of information concerning drugs pharmacologically active at the neuromuscular junction has accumulated during the past few years. Very often, however, findings and interpretations differ. Heterogeneity of experimental techniques and the use of various muscle preparations are likely to explain at least in part these discrepancies. For example, if all the various results are put together one cannot help reaching the conclusion that while there is a good agreement between the results obtained in vivo, from muscles with their natural circulation, hazards accompany the study of a muscle in vitro (Zaimis 1964).

Maclagan (1962) studied the tenuissimus muscle of the cat both in vivo and in vitro and found that the uniformity and predictability of the response was lost when the muscle was studied in vitro. For example she reported that while tubocurarine effectively antagonized the early doses of decamethonium its effectiveness decreased when administered during subsequent blockades, a situation totally different from that recorded in vivo. Thus the effectiveness of tubocurarine as an antagonist could be correlated with the number of times that the muscle had been treated with decamethonium. After several doses of the depolarizing drug and at a stage when the muscle appeared to be in good condition, tubocurarine antagonism occurred in only 15–20% of the occasions on which it was tested.

Moreover, the frequency with which the nerve is stimulated, or disturbances in the acid-base balance of the body, can affect the action of tubocurarine. The higher the frequency, the greater the degree of block. Acidosis prolongs its action while alkalosis antagonises it.

Although the fundamental concept of the neuromuscular "junction" was provided by an observation made by Pelouze and Claude Bernard in 1850, only the past 30 years have witnessed remarkable progress in all areas of muscle physiology and pharmacology. But even so, many aspects are still veiled in complete mystery. It is, therefore, most satisfactory that the necessity for collaboration between different scientific disciplines is becoming more widely recognized. But this collaboration must not stop at

mere exchange of information; it needs to be pursued through combined experimentation and interdisciplinary team work.

Summary

The ability of a given dose of tubocurarine to produce a large or a small effect depends on the ease with which the drug can compete with acetylcholine (ACh). The duration of the blockade, however, is governed primarily by factors such as the rate of excretion and destruction of tubocurarine which determine how long and in what concentration this drug is present at the neuromuscular junction. For example, in experiments in which the cumulative effects of tubocurarine are minimised, a lowered muscle temperature reduces the magnitude of the tubocurarine blockade but has little influence on the duration of its action.

Besides the effect of temperature, other factors such as age, sex, denervation, disuse atrophy and hypertrophy have been adduced to account for changes in the action of tubocurarine. Moreover the frequency with which the nerve is stimulated or disturbances in the acid-base balance of the body can also affect the action of tubocurarine. Finally, heterogeneity of experimental techniques and the use of isolated muscle preparations are likely to explain part of the discrepancies found during the study of neuromuscular blocking drugs.

The evidence so far available for this range of variables affecting the action of curare is discussed.

Zusammenfassung

Das Vermögen einer bestimmten Dosis von Tubocurarin, eine geringere oder stärkere Wirkung auszulösen, hängt davon ab, inwiefern die Droge mit dem Acetylcholin in Konkurrenz treten kann. Die Dauer der Blockierungswirkung wird indessen primär von der Schnelligkeit der Ausscheidung und des Abbaus des Tubocurarins beeinflußt. Diese Faktoren entscheiden, wie lange und in welcher Konzentration das Tubocurarin in den neuromuskulären Synapsen haftet. In Experimenten zum Beispiel, in denen der Kumulativeffekt von Tubocurarin stark vermindert wird, reduziert eine Senkung der Muskeltemperatur wohl den Grad der durch Tubocurarin hervorgerufenen Blockierung, hat aber auf die Dauer der Wirkung dieses Stoffes nur geringen Einfluß.

Außer der Temperatur wurden auch andere Faktoren beobachtet, welche die Wirkung des Tubocurarins verändern, so z. B. das Alter und das Geschlecht, die Denervation, ferner Atrophien infolge Untätigkeit und Hypertrophien. Außerdem können die Frequenz der Nervenreizung oder auch Störungen im Säure-Basen-Gleichgewicht des Körpers die Wirkung von Tubocurarin beeinflussen. Und schließlich erklären wohl auch die Heterogenität der experimentellen Methoden und die Verwendung isolierter Muskelpräparate einen Teil der Diskrepanzen, denen man beim Erforschen der neuromuskulären Blockierungsdrogen begegnet.

Das bereits vorliegende Material über die ganze Reihe von Wirkungsfaktoren in der Anwendung von Tubocurarin wird diskutiert.

Résumé

L'efficacité d'une dose donnée de tubocurarine à produire un effet marqué ou léger dépend de la facilité avec laquelle la drogue peut entrer en compétition avec l'acétylcholine (ACh). La durée du blocage, cependant, est primordialement fonction de facteurs tels que la vitesse d'excrétion et de destruction de la tubocurarine, facteurs qui déterminent pendant quelle durée et à quelle concentration la drogue est présente à la jonction neuromusculaire. Par exemple, lors d'expériences où les effets cumulatifs de la tubocurarine sont minimisés, un abaissement de la température musculaire réduit le degré du blocage produit par la tubocurarine, mais a peu d'influence sur sa durée d'action.

Outre l'effet de la température, d'autres facteurs, tels l'âge, le sexe, la dénervation, l'atrophie par inactivité et l'hypertrophie, peuvent causer des changements dans l'action de la tubocurarine. De plus, la fréquence par laquelle le nerf est stimulé, ainsi que des perturbations de l'équilibre acido-basique de l'organisme, peuvent également affecter l'action de la tubocurarine. Finalement, l'hétérogénéité des techniques expérimentales et l'emploi de préparations de muscles isolés peuvent rendre compte en partie des discordances notées lors de l'étude des médicaments bloqueurs neuromusculaires.

Les preuves, dont nous disposons jusqu'à présent dans ce domaine des variantes affectant l'action de la tubocurarine, sont discutées.

Riassunto

La facoltà di produrre un effetto più o meno potente che una determinata dose di tubocurarina può avere, dipende dalla facilità con cui questa droga può entrare in competizione con l'acetil-colina. La durata del blocco invece, è prima di tutto funzione di fattori quali la velocità d'escrezione e di distruzione della tubocurarina, fattori che determinano per quanto tempo ed in quale concentrazione la droga è presente al livello della congiunzione neuromuscolare. Per esempio, durante esperimenti in cui gli effetti cumulativi della tubocurarina sono minimizzati, un abbassamento della temperatura muscolare diminuisce il grado di blocco prodotto dalla tubocurarina, ma influenza poco la sua durata d'azione.

Oltre all'effetto della temperatura altri fattori quali l'età, il sesso, la denervazione, l'atrofia da inattività e l'ipertrofia, possono causare dei cambiamenti nell'azione della tubocurarina. Inoltre la frequenza con cui il nervo è stimolato, come pure dei perturbamenti nell'equilibrio acido-basico dell'organismo, possono ugualmente influenzare l'azione della tubocurarina. E per terminare, l'eterogeneità delle tecniche sperimentali e l'uso di pre-

parati quali i muscoli isolati, possono spiegare in parte le differenze notate studiando i medicamenti ad azione neuromuscolare bloccante.

Cercheremo di esaminare le diverse varianti capaci di influenzare l'azione del curaro.

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