pH AS A FACTOR INFLUENCING PLASMA CONCENTRATIONS OF d-TUBOCURARINE

Preliminary Communication

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SUMMARY

A series of experiments into plasma concentrations of d-tubocurarine in the dog is described. It was found that the higher the blood pH at which distribution of the drug was taking place the lower were the plasma concentrations, and vice versa. The plasma concentrations diminished when the blood pH was raised during the distribution of the drug but it was not found possible to demonstrate an increase in plasma concentrations when the blood pH was lowered. This relationship between plasma concentrations and pH was apparently not mediated by a renal mechanism. The significance of these findings is discussed.

Investigations into the plasma concentrations of d-tubocurarine found after the intravenous administration of the drug (Mahfouz, 1949; Pittinger, Morris, and Cullen, 1951; Marsh, 1952; Cohen, Paulson and Elert, 1957; Aladjemoff, Dickstein and Shafrir, 1958) have shown that the decline in the plasma concentrations takes place in two phases, an initial phase in which the rate of decline is rapid being followed by a phase in which the rate of decline is much slower. Despite this qualitative similarity between the various accounts, however, there is little or no correlation between the dose of the drugs given and the actual plasma concentrations found and no satisfactory explanation has been offered for this surprising discrepancy.

Work by the author on anaesthetized patients given large doses of the drug suggested that blood pH might be a factor influencing plasma concentrations. It was, however, found impossible to pursue this line of inquiry in the operating theatre and a laboratory investigation in dogs was undertaken, the dog being the only available laboratory animal from which the large samples of blood (12 ml) required for the accurate determination of d-tubocurarine could be taken. This paper gives a preliminary account of the results of this work.

METHOD

Determination of plasma concentrations of d-tubocurarine.

The method used (with some modification) was that of Elert (1956) which is based on the ultra-violet spectrophotometric determination of the drug. This gives a measure of total d-tubocurarine (i.e. "free" drug plus any bound to plasma protein). The d-tubocurarine is extracted from plasma into ethylene dichloride at alkaline pH in the presence of potassium iodide (the method of Quinn and Woislawski, 1950). Subsequently the alkaloid is re-extracted into 0.01 N hydrochloric acid and estimated at 280.5 m\(\mu\) in the spectrophotometer (in this study the wavelength used was 280 m\(\mu\)).

Five-ml samples of plasma were added to 10 ml of ethylene dichloride (purified by repeated washings with dilute hydrochloric acid); 1 ml of freshly prepared potassium iodide-glycine buffer (Quinn and Woislawski, 1950) was then added, the mixture shaken by hand for 5 min, transferred to a centrifuge tube and spun at 4,000 r.p.m. for 5 min. The upper (aqueous) phase was then discarded and 5 or 7 ml of the organic phase added to 5 ml of 0.01 N hydrochloric acid. The mixture of acid and ethylene dichloride was shaken for 5 min, transferred to a centrifuge tube and spun at 2,000 r.p.m. for 5 min. The acid layer was then drawn off with a pipette and placed in a glass tube. To remove any remaining organic solvent a current of air was blown through the acid which was then ready for spectrophotometry. The spectrophotometer used was a Unicam SP500.

Basically the OD\(_{280}\) of the acid solution.
obtained from extraction of a sample of plasma containing d-tubocurarine has to be read against a sample of acid derived from the same plasma to which no d-tubocurarine has been added. This sample, the plasma “blank”, gives a measure of the optical density at 280 m\(\mu\) of those substances other than d-tubocurarine which have been extracted into the acid from the plasma or which are derived from the reagents.

To test the validity of the method, work was carried out on serial dilutions of d-tubocurarine at first in water and later in plasma, the samples in water being treated in the same way as those in plasma, using a water blank. For this work a series of standard solutions of d-tubocurarine was made up in water after the method of Klein and Gordon (1949). The results from six serial dilutions in plasma are shown in figure 1. As the volume of ethylene dichloride used in the second stage was either 5 or 7 ml, the results of the optical density measurements were multiplied by 10/5 or 10/7 respectively to facilitate comparison (the “corrected optical density”).

In all the experiments on serial dilutions in plasma the maximum error in 37 samples of 10 \(\mu\)g/ml or less was 0.7 \(\mu\)g/ml and the error tended to be less at the lower concentrations.

In most of the plasma samples obtained from dogs the concentrations found were less than 5 \(\mu\)g/ml.

In the experimental work on plasma concentrations in dogs the plasma “blank” was duplicated since any error in this measurement makes the readings worthless. In addition, three samples of d-tubocurarine in water of known concentration were put through the stages of the method to ensure that there had been no change in the relationship between concentration of d-tubocurarine and optical density such as might occur due to an instrumental change in the spectrophotometer. In practice it was found that the relationship remained the same. Recoveries were performed in about half the experiments and varied from 97 to 105 per cent.

To ensure good results sedulous attention has to be paid to cleaning glassware, preparing the reagents, and, above all, to avoiding contamination during the collection of specimens.

**Experiments on dogs.**

Experiments were performed on eighteen dogs. They were anaesthetized with a small intravenous dose of thiopentone (this drug does not interfere with the estimation of d-tubocurarine) and the trachea was intubated. Anaesthesia was maintained with a mixture of nitrous oxide and oxygen, pulmonary ventilation being provided by a Palmer “Ideal” respirator. Each animal was ventilated at the same rate (28/min) but the tidal volume was adjusted to be roughly proportionate to the weight of the animal. The pulmonary ventilation used was high and always resulted, other things being equal, in the development of a respiratory alkalosis. When it was desired to investigate the lower ranges of blood pH, carbon dioxide was added to the inspired mixture.

A constant dose of 0.8 mg/kg of d-tubocurarine was used in every experiment. The drug was administered through a cannula in the femoral vein. Arterial blood pH, carbon dioxide tension and standard bicarbonate were estimated by the method of Robinson and Utting (1961), blood being withdrawn from a cannula in the femoral artery. Samples for the estimation of d-tubo-
curarine were taken from a cannula in the other femoral artery.

The dog is not an ideal animal for this type of research since it develops a precipitous fall of blood pressure when d-tubocurarine is given, apparently due in large part to release of histamine. With two exceptions, however, the response of the animals was sufficiently uniform to make interpretation of the results straightforward.

Because of the large samples of blood required for the accurate determination of plasma concentrations of d-tubocurarine, it was necessary to study the rapid phase of decline and the early part of the slow phase in some animals, and in others to confine attention entirely to the slow phase (fig. 2).

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RESULTS

The fall of plasma concentrations of d-tubocurarine in the dog, as already shown in the human, takes place in at least two phases, an initial phase in which the decline is rapid being followed by a phase in which the decline is much slower (fig. 2). The plasma concentrations vary according to the pH at which the experiment is conducted. In general, when the pH is high the plasma levels are low and vice versa. Inspection of figure 2 shows that the plasma concentrations at pH 7.2 are several times greater than those at pH 7.6. The relationship is not, however, linear.

![Diagrammatic representation of the findings relating plasma concentrations of d-tubocurarine and blood pH during the slower phase of distribution based on 69 determinations in 15 dog experiments.](https://academic.oup.com/bja/article-abstract/35/11/706/276934)

In the initial phase the changes of plasma concentration take place too rapidly to permit of adequate investigation and it is only in the slower phase of decline of plasma concentration that the relationship between pH and plasma concentrations can be clearly seen. Figure 3 is an attempt to show the relationship between pH and plasma concentration during the slow phase of the fall in plasma levels. Two experiments have been excluded since the response of the animals to the injection of the drug was quite atypical, the fall in the blood pressure being only a small fraction of that found in the others. Also excluded are those values for plasma concentration obtained after a
change of pH had been induced in the animal. There are left for comparison seventy-four estimations and all but five of these fit into the compartments shown.

In three out of the four experiments in which the blood pH was raised during the slow phase of distribution by turning off the carbon dioxide in the inspired gas mixture there was a sudden and considerable fall in plasma concentrations. In the other experiment the increase in pH was too small to permit of any conclusion. This fall in plasma concentration when the blood pH was raised still took place in an animal which had been subjected to bilateral nephrectomy (fig. 4). On the other hand, it was not found possible in the two experiments in which it was attempted to demonstrate an increase in plasma concentrations when the pH was decreased by adding carbon dioxide to the inspired gas mixture during the slow phase of distribution.

In most of the experiments the arterial blood pressure remained too low for the kidneys to function for the first half-hour or more after the drug had been given. Results from those animals which had been subjected to bilateral nephrectomy, however, seemed to indicate that the kidneys were responsible for eliminating some of the drug when the blood pressure had reached a level compatible with renal function. A full account of this work will be given later.

DISCUSSION

There are good theoretical reasons for supposing that pH might influence plasma concentrations of d-tubocurarine despite the fact that changes in pH within the biological range have relatively little effect on the drug molecule itself; the pK values of the quaternary ammonium groups are very high and those of the phenolic hydroxyl groups are also high, being 8.1 and 9.1 (Kalow, 1954). On the other hand, there is evidence that d-tubocurarine is bound to the plasma protein (Aladjemoff, Dickstein and Shafrir, 1959) and such binding of drug to plasma protein is influenced by pH, usually being diminished in alkalosis (Goldstein, 1949).

When considering the effect of pH on drug action it is important to remember that pH may have an effect on the receptors for the drug as well as the drug itself (Albert, 1952). Recent work on curare and curare-like compounds has shown that the distribution of these drugs in the body is widespread, not merely confined to the neuromuscular junction, and that the receptors in the body for these substances are possibly mucopolysaccharide acids (Chagas, 1962; Cavalli, 1962). It is possible that the main effect of change in pH on plasma concentrations of d-tubocurarine is due to changes in the receptor substance. Increase in the pH of the blood and body fluids may increase the degree of ionization of the receptors not only at the neuromuscular junction but also elsewhere and thus lead to a diminution of the plasma concentration of the drug.

It is not claimed that a relationship between pH and plasma concentrations of d-tubocurarine has been proved in the human subject but the results obtained suggested that the relationship might be the same as that which was found in the dog. As a low plasma concentration is likely to be associated with a small degree of neuromuscular block this suggests that pulmonary hyperventilation might diminish the degree of block in an anaesthetized patient. Payne (1958) found that carbon dioxide increased the degree of block due to d-tubocurarine in the cat, but subsequently (1960) found that the production of a metabolic acidosis in the animal diminished the degree of block.

Pulmonary hyperventilation in anaesthesia, in which d-tubocurarine is used tends to stop muscular movements. This may seem surprising if
hyperventilation leads to a lesser degree of neuromuscular block than would be obtained if hyperventilation were not practised. It is, however, quite possible that hyperventilation minimizes muscular movements by a central action, and that this is purchased at the expense of diminishing the degree of effectiveness of the relaxant, an idea finding some support in the fact that those who practise passive pulmonary hyperventilation commonly use large doses of the drug. Even with these large doses, reversal of the neuromuscular block by neostigmine is prompt and effective; this may be due to an increased elimination of the drug by mechanisms other than renal under conditions in which the pH is high.

REFERENCES


L'INFLUENCE DU pH SUR LES CONCENTRATIONS DE D-TUBOCURARINE DANS LE PLASMA

SOMMAIRE

Description d'une série d'expériences faites sur le chien, de dosage des concentrations plasmatiques de d-tubocurarine sous l'influence de divers degrés de pH. L'auteur trouva que plus le pH sanguin est élevé au moment de la diffusion du produit, plus les concentrations plasmatiques de d-tubocurarine étaient basses et inversement. Les concentrations plasmatiques diminuèrent lorsque le pH sanguin était augmenté au moment de la diffusion du produit, mais l'auteur ne réussit pas à en démontrer l'augmentation dans sa concentration plasmatique après réduction du pH sanguin. Ce rapport entre concentrations plasmatiques et pH n'eut apparemment pas lieu par intermédiaire des reins. L'auteur discute la signification de ces constatations.

pH ALS EIN FAKTOR MIT EINFLUSS AUF DIE PLASMAKONZENTRATIONEN DES D-TUBOCURARINS

ZUSAMMENFASSUNG