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To Awake a Sleeping Beauty

John Wallwork B.Sc.

Abstract

Every year over 1,000 hospital admissions for accidental poisoning, attempted suicide, or drug overdosage occur in this city and there is no doubt that the size of this problem is increasing.

A large variety of drugs are taken and the specific treatment of the more common poisonings is adequately dealt with in an excellent monograph of Mathew and Lawson. It will help however to consider some general principles of how to tackle the acute problem clinically. You can:

(1) Prevent the drug entering the body's circulation (stomach washouts)
(2) Prevent the drug reaching its site of action once in the body
(3) Prevent the drug from exerting its effect at this site by
    (a) direct competitive inhibition
    (b) providing alternative metabolic pathways to overcome drug action
(4) Enhance the rate of drug metabolism
(5) Remove the drug from body— (diuresis, dialysis).

Of these methods, the first, and the last to a lesser extent, is widely used in practice in this country. Again I refer you to Mathew's book for the details.

I am interested in the third of these, that is to prevent the drug from exerting its effect at its site of action by providing alternative metabolic pathways, with particular reference to barbiturate poisoning, which constitutes the largest single group of agents encountered in clinical practice.

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Before we can antagonise a drug's action in this way we must know where that site of action is, and how the drug works.

Let us look at a little simple biochemistry. The main biochemical pathway for the production of energy is the metabolism of glucose. Represented in a simplified form.

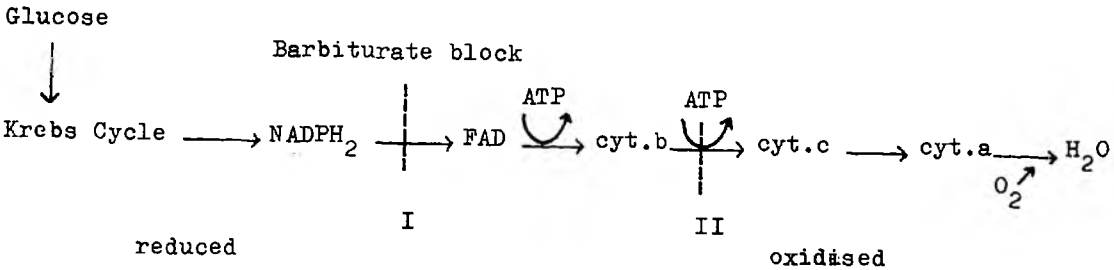
Glycolysis → Krebs cycle → E.T. Chain → Energy.

Theories concerning the mode of action of barbiturates on this system have varied. Early work by Brody and Bain suggested that the action of barbiturates was mediated by an uncoupling of oxidative phosphorylation. However Aldridge and Parker (1960) showed that inhibition of respiration occurred without uncoupling, in liver mitochondria; more important from our point of view, they also showed that the inhibition produced by barbiturates on the respiration of mitochondria, did not occur when succinate was used as substrate instead of glucose. It was concluded that the block must occur somewhere before the entry of succinate into the metabolic process.

Elaborate techniques used by Chance and Hollinger in 1965 corroborated this work.

Imagine the process as a production line with substrate in a reduced form at the left and products in oxidised forms to the right. If we stop this continuous process somewhere in the middle, there will be a build up of reduced substances to the left and a depletion of oxidised substances to the right of the block. The substances involved can be measured in both their oxidized and reduced state in the presence of a barbiturate in the metabolic pathway. Therefore the site of block in the chain can be determined by the point at which the transition between elevation of the reduced proportion and depletion of the oxidized proportion of such substances occurs. This is then the site of action of the barbiturate.

Chance and Hollinger showed a build up of reduced $NADPH_2$ and a depletion of oxidized cytochrome b in the presence of barbiturate and concluded that its site of action must be between $NADPH_2$ and FAD cyt. b (Site I in diagram). If the site of action had been site II then they would have observed an accumulation of reduced cyt. b and depletion of oxidized cyt. c.



It can be concluded that barbiturates prevent respiration of liver mitochondria by inhibition of the metabolism at the site shown ($NADPH_2$/cyt. b).

This is very satisfying biochemically, but all this work was done in vitro, a long way from the complicated structure and mechanism of whole brain. Does barbiturate have the same action in whole brain?

We designed an experiment on the hypothesis that barbiturates have the same site of action in vivo and in vitro. It involved anaesthetising rats with a barbiturate and giving them a substrate, metabolised in brain, which enters the pathway before the proposed site of action (we used pyruvate), and one that enters after the proposed site of action (we used succinate). We observed.

This sounds easy. However, the difficulties in administering substances to the brain in known concentration are great. The substances we used, pyruvate, succinate, are utilized by other tissues in the body; they are also highly ionized and therefore will not easily cross the so-called blood-brain barrier.

Recently new techniques have been developed to overcome such difficulties, some of great sophistication; we used a rather crude but effective technique.

Once the rat is anaesthetized by an intraperitoneal injection of barbiturate, a small hole is bored at a particular point on the skull. Substances are then injected in small volumes into one of the lateral ventricle of the brain, through a special needle so designed that the depth from guard to tip is the depth required to reach the lateral ventricle in rats of a certain weight.

Using this technique the experiment was elaborated using three groups of rate of the same age, sex and weight. One group received standard dose of barbiturate alone, one group barbiturate and intraventricular succinate and the other group barbiturate and intraventricular pyruvate. The sleeping times of all rats, that is the time from onset of anaesthesia to time of arousal, were determined.

Other variables were eliminated by using

(1) Rats of the same strain and weight and receiving a standard dose of barbiturate I.P.
(2) Starved rats, so that absorption from peritoneum would be more predictable.
(3) Male rats since Brody has shown that barbiturates have different lengths of action in different sexes.

The results were striking. There was no significant difference in the control group and the group given pyruvate; both groups having sleeping times of around 100 minutes. However, the group receiving succinate had a mean sleeping time of around 30 minutes. This is a highly significant difference.

It can be argued that succinate in some way decreased the concentration of available barbiturate either by increasing its metabolism or by inactivating it. In order to eliminate this possibility, samples of blood were collected from all rats by decapitation at the time of arousal. The blood barbiturate levels were estimated. The levels in the blood of the succinate group killed at around 30 minutes were found to be significantly higher than the levels in the control and pyruvate group killed around 100 minutes. This was to be expected if the succinate did not affect the barbiturate blood level and it was concluded that the

action of succinate must be central in the brain. (Intra cellular oxidation at reversal level).

From these results we can postulate that our original hypothesis was correct since, like the mitochondria discussed earlier, the rats could utilize succinate for energy production, to overcome the barbiturate anaesthesia, but were not able to utilize pyruvate. We therefore concluded that the barbiturates act in the similar way in whole brain as they do in isolated mitochondria.

The question now arises as to whether the action of barbiturates cannot be reversed in humans, if succinate can reverse barbiturate anaesthesia in rats. This seems to be a good idea, but I have already mentioned difficulties due to blood-brain barrier effects, which would exclude the possibility of using succinate intravenously. In fact two workers, Soskin and Taubenhous, tried this, with no effect, in 1943, long before Kregs invented his cycle.

It is difficult to persuade anybody that boring holes in human heads to inject directly into brain tissue is of justifiable therapeutic value. And the present mortality rate after hospital admission is small, due to the extensive supportive therapy employed in overdose treatment.

It has been suggested that we perform cysternal punctures in particularly ill patients but this is a difficult and not widely practiced technique and would be available only to the few.

We must use an intravenous route of administration if this method of treatment is to be useful and we must therefore modify the succinate molecule in some way. It occurred to us that the answer might be to make a fat-soluble succinate molecule with the active site free. It could enter the brain and still act as a substrate for metabolism. A substance that appeared to have the required physical properties was monoethylsuccinate, highly soluble in water and organic solvents.

$CH_2 — COOH$	$CH_2 — COCH_5$
$CH_2 — COOH$	$CH_2 — COOH$
succinate	monoethylsuccinate

Finding a recipe to make this substance proved to be more difficult. However, we now have a whole 50 grams and hope to begin work soon. The theory is that this substance will enter brain in sufficient concentrations and will be split by esterases to give free succinate and, incidentally, ethyl alcohol to bring you round drunk.

As I have already indicated, barbiturate poisoning is a major problem. Much time and money and many lives could be saved if every doctor in the land could wake these sleeping beauties with a simple injection.

There is a long way to go. This is only the beginning.

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