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## PRELIMINARY COMMUNICATION

# REGIONAL DISTRIBUTION IN BRAIN AND EFFECT ON CEREBRAL MITOCHONDRIAL RESPIRATION OF THE ANTICONVULSIVE DRUG n-DIPROPYLACETATE

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It is well known that anticonvulsive drugs such as barbiturates and hydantoin lower the level of cellular respiration[1,2] with resultant general depressive effects on behaviour. It thus seemed pertinent to search for drugs acting in a more direct physiological fashion, e.g. by raising the cerebral level of a natural inhibitory transmitter, γ-aminobutyric acid (GABA). Administration of GABA itself does not produce a significant increase in the cerebral level, as this substance does not normally traverse the blood brain barrier. An increase in brain GABA level can be obtained by administration of amino-oxy-acetic acid (AOAA)[3,4], but this substance inhibits all pyridoxal phosphate linked enzymes and is thus highly toxic to the organism.

Since the anticonvulsive properties of n-dipropylacetate (nDPA) were first demonstrated by Carraz *et al.*[5], Godin *et al.*[6] have shown that administration of this drug to rats and mice bring about an increase in the cerebral level of GABA. Using mice (Swiss albinos, Rb strain) sensitive to audiogenic seizure, Simler *et al.*[7] have shown that after administration of nDPA there is a parallelism between the protective effect against acoustically induced convulsions and the increase in the cerebral GABA level. *In vitro*, this compound has been found to be an inhibitor of cerebral GABA transaminase, competitive with one of its substrates : GABA[8,9]. The major part of nDPA remains unchanged[10] in the brain for the duration of its pharmacological action. Thus, there is a good probability that it acts by a specific mechanism, i.e. the inhibition of cerebral GABA transaminase with a resultant increase in GABA level. Moreover, nDPA does not cause adverse behavioural side effects, in fact the avoidance conditioning response is somewhat improved[13].

In this work we first compare the effects of nDPA and phenobarbitone on mitochondrial respiration, then using audiogenically sensitive mice of the type employed by Simler *et al.*[7], we study the relationship between the time courses

TABLE 1. Cerebral mitochondrial respiration

| Compound added | $O_2$ Uptake (µl $O_2$/min/mg protein) |
|---|---|
| Pyruvate 2.9 mM | 0.18 ±0.01 (n = 4) |
| Pyruvate 2.9 mM + ADP 0.7 mM | 0.25 ±0.02 " |
| nDPA 2.9 mM | 0.13 ±0.01** " |
| nDPA 2.9 mM + ADP 0.7 mM | 0.21 ±0.02** " |
| nDPA 5.8 mM + pyruvate 2.9 mM | 0.28 ±0.03** " |
| nDPA 23.2 mM + pyruvate 2.9 mM | 0.46 ±0.02** " |
| Phenobarbitone 0.58 mM + pyruvate 2.9 mM | 0.11 ±0.01** " |
| Phenobarbitone 1.1 mM + pyruvate 2.9 mM | 0.09 ±0.01** " |

**$P < 0.005$.
The incubation medium was that of Bradford *et al.*[11]. Oxygen uptake was measured at 37°C for 15 min with a Clark type electrode. The measurements are expressed as µl of oxygen uptake per mg protein per min compared to that of the control (100 %) using 2.9 mM pyruvate as substrate. The concentrations of 5 mM and 20 mM nDPA correspond to those found in mouse brain homogenate after injection of 100 mg/kg and 400 mg/kg $^{14}$C-nDPA respectively. Similarly, the phenobarbitone concentrations used correspond to the values found in mouse brain homogenates by Mazel *et al.*[12] after injection of 234 mg/kg and 468 mg/kg. The latter value corresponds to 2.5 times the median anaesthetic dose.

of protection against seizure, the increase in cerebral GABA level and the extent of nDPA fixation in the brain. Finally using rats, we compare the regional levels of nDPA fixation with the levels of GABA transaminase found in these same brain regions by Robinson and Wells[14].

It can be seen (Table 1) that nDPA raises the level of respiration significantly, when added to mitochondria, using 2.9 mM pyruvate as substrate. Moreover, nDPA supports respiration alone, and this respiration is increased to the

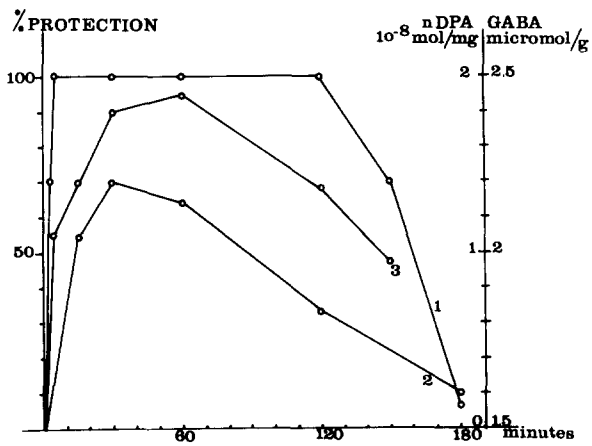


FIGURE 1. Curves 1 and 2 show the time course of protection against audiogenic seizure and the change in cerebral GABA level respectively after injection of 400 mg/kg nDPA into audiogenetically sensitive Swiss albinos mice, Rb strain. Curve 3 shows the progress of cerebral fixation of $^{14}$C-nDPA after intraperitoneal injection of 400 mg/kg + 50 µCi/kg of this drug into the same strain of mice. To measure fixation of $^{14}$C-nDPA, the animals were decapitated at times 5, 15, 30, 60, 120 and 150 min after the injection. The brains were rapidly removed, weighed and homogenised in 6 M NaOH. After digestion at 60°C, one aliquot was taken for protein determination by Lowry *et al.*'s method[15] and another for liquid scintillation counting.

TABLE 2. Regional nDPA fixation

| | nDPA fixation µmoles/g wt wt | GABA transaminase activity |
|---|---|---|
| 1. TELENCEPHALON | | |
| Neocortex, superior | 5 | ++ |
| Caudate nucleus and Putamen | 50 | ++++ |
| Globus pallidus | 50 | ++ |
| Nucleus accumbens septi | 55 | ++++ |
| Hippocampus | 25 | ++ |
| Body of fornix | 75 | ++ |
| Internal capsule | 5 | - |
| Corpus callosum | 5 | - |
| Neocortex, lateral | 11 | ++ |
| Neocortex, posterior | 5 | † |
| Commisura anterior | 135 | † |
| 2. DIENCEPHALON | | |
| Thalamus | 7 | ++ |
| Hypothalamus | 7 | ++ |
| Lateral preoptic area | 15 | ++ |
| 3. MESENCEPHALON | | |
| Reticular formation | 15 | +++ |
| Substantia nigra | 15 | +++ |
| Red nucleus | 15 | +++ |
| Nucleus darkschevitch and IV | 10 | + |
| Pedonculus | 25 | † |

++++ intense ; +++ strong ; ++ moderate ; + weak ; - absent ; † GABA transaminase activity not determined. To measure nDPA, Wistar rats were injected intraperitoneally with 100 mg/kg + 200 µCi/kg $^{14}$C-nDPA. After 45 min, the time corresponding to maximum pharmacological activity of this drug in mice, the rats were decapitated and the brains rapidly removed and frozen at -25°C. They were cut into 1 mm sections and each was then dissected under a binocular lense. The resulting fragments were placed in tared scintillation vials, weighed, then solubilized in soluene 100 ® prior to liquid scintillation counting.

same extent by addition of 0.7 mM ADP as by addition of ADP to mitochondria using pyruvate as substrate.

Figure 1 shows the time course of nDPA fixation to the mouse brain after intraperitoneal injection. On the same graph are shown the results of Simler *et al.*[7] with respect to protection against seizures and cerebral GABA level.

One can seen that maximum nDPA fixation occurs at the height of its pharmacological activity, i.e. when protection against audiogenic seizures is 100 % and when the GABA level is at its maximum.

Table 2 compares the GABA transaminase activity in various regions of the rat brain found by Robinson and Wells[14] with the level of nDPA fixation in these regions investigated in our laboratory.

It can be seen that a higher level of fixation of nDPA takes place in those regions of the brain displaying high GABA transaminase activity. There is a much higher level of nDPA fixation in the striatum, septum and fornix where GABA metabolism is particularly active than in the cortical regions where there is little activity. The method used was a quantitative one, but the dissection was perfor-

med on rather large areas compared to those defined by histochemical means. Thus the correlation is not always very strict since the distribution pattern was done by histochemical methods which provide data on very small areas, these data being semi quantitative.

These fixation studies illustrate in the first place a direct relationship between nDPA fixation, increase in GABA level and protection against experimentally induced convulsions, and in the second place that the degree of nDPA fixation to various brain regions is directly related to the GABA transaminase activity found in these same areas. Thus it may be postulated that GABA transaminase is the receptor for nDPA which being a GABA analogue, inhibits the enzyme competitively with respect to GABA, causing an elevation in the level of this natural neurotransmitter. However, a direct effect by nDPA on the GABA synaptic receptor cannot be ruled out. Research is in progress into other compounds which may cause an elevation of cerebral GABA level by specific inhibition of GABA transaminase in parallel to protection against a convulsive state.

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