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Inhibitory processes in the brain

Inhibitory influences in the brain were first demonstrated by Bubnoff and Heidenhain as early as 1881[1], and in 1931, Hess claimed that 'the essential mechanism of sleep cannot be explained differently as by active inhibition of some functions of the organism' [2]. Today, we know that inhibitory processes are as important as activating influences for brain functioning.

Transmitters are either activating or inhibitory, according to their target structure. Acetylcholine, which is more often activating, also has inhibitory influences, in the thalamic reticular nucleus [3], for example. Dopamine, noradrenaline, serotonin (5-HT) and histamine have mainly inhibitory influences at cortical level [4–7]. All of these transmitters have a common characteristic: they act using a metabotropic mechanism that involves the complex functioning path of protein G (except when acting on 5-TH_3 receptors). Their influence might be judged as diffuse because they are most often released at varicosities level [8] and, therefore, are often considered as neurohormones. These transmitters have a mainly regulating influence. However, a specific inhibitory neurotransmitter is present in the brain, except during the first week of life, namely, gamma aminobutyric acid (GABA). The history of this neurotransmitter is long, beginning discretely in 1862, when barbiturates were synthesized, and overtly in 1903, with their first clinical application. The demonstrated efficacy of GABA in anesthesia, epilepsy and insomnia gave rise to extensive research, which led progressively to the discovery of the $GABA_A$ receptor and its various binding sites. All of the sites, except the picrotoxin binding-site, when activate, decrease waking. However, one site has additional anxiolytic potential, with the discovery of tranquillizers acting at the benzodiazepine binding-site. Unlike the previous transmitters, the $GABA_A$ receptor largely works via the simpler ionotropic mechanism – the binding of the transmitter directly induces the opening of a Cl^- channel, leading to neuron hyperpolarization.

The story continued with the identification of the $GABA_B$ receptor [9], the only GABA metabotropic receptor. Its influence on vigilance, via Ca^{2+} and/or K^+ ion channels, was similar to $GABA_A$ receptor; but, paradoxically, it was shown that antagonists have therapeutic indications in absence epilepsy [10].

Finally, identification of the $GABA_C$ receptor added an important new piece to our knowledge of inhibitory processes in the brain. This receptor also involves the Cl^- ion channel, but its sensitivity to GABA is significantly higher than both previous receptors and its desensitization is much slower, at least, than the $GABA_A$ receptor [11]. Consequently, when specific agonists become available for clinical use, they could be effective for the treatment of epilepsy and insomnia, while antagonists could be helpful in narcolepsy; they are effective at lower doses and, therefore, have fewer side effects.

For decades, we have studied the influence of GABAergic compounds on sleep–waking mechanisms. Today, it can be said that agonists of the three types of receptors do induce sleep [12]; however, they have a distinct influence in one particular stage of sleep: paradoxical or rapid eye movement (REM) sleep, during which, dreaming occurs. $GABA_A$ agonists [13] inhibit and $GABA_B$ antagonists favor this sleep stage [14], whereas, $GABA_C$ receptor antagonists shorten it [15] and agonists favor it (research in progress). It seems that, whereas $GABA_A$ and $GABA_B$ receptors are situated in what is called 'paradoxical sleep-on' generating structures (principally cholinergic and glutamatergic) [16], $GABA_C$ receptors are located on structures that prevent the occurrence of this stage [12] (i.e. paradoxical sleep-off 'permissive' structures that are serotonergic [16] and noradrenergic [17]). Consequently, the progressive increase in GABA mechanisms knowledge already demonstrates a large spectrum of brain influences.

References

1 Bubnoff, N. and Heidenhain, R. (1881) Ueber erregungs-Hemmungsvorgänge innerhalb der motorischen Hirncentren. In: Pflüger, E.F.W. Ed. pp. 137–202. *Arch. Gen. Physiol. Emile Strauss Verlag*

2 Hess, W.R. (1931) Le sommeil. *C R Soc Biol* 107, 1333–1364

3 Steriade, M. and McCarley, R.W. (1990) *Brainstem control of wakefulness and sleep.* Plenum Press

4 Krnjevic, K. and Phillis, J.W. (1963) Actions of certain amines on cerebral cortical neurons. *Br. J. Pharmacol.* 20, 471–90

 1359-6446/03/$ – see front matter PII: S1359-6446(03)02842-3

5 Reader, T.A., *et al.* (1979) Modulatory role for biogenic amines in the cerebral cortex. Microintophoric studies. *Brain Res.* 160, 217–229
6 Manunta, Y and Edeline, J.M. (1999) Effects of noradrenaline on frequency tuning of auditory cortex neurons during wakefulness and slow wave sleep. *Eur. J. Neurosci.* 11, 2134–2150
7 Schwartz, J.C. *et al.* (1991) Histaminergic transmission in the mammalian brain. *Physiol. Rev.* 71, 1–51
8 Levit, P. and Moore, R.Y. (1978) Noradrenaline neuron innervation on the neocortex in the rat. *Brain Res.* 139, 219–232
9 Hill, D. R and Bowery, N.G. (1981) ^{3}H-baclofen and ^{3}H-GABA bind to bicuculine insensitive $GABA_A$ sites in rat brain. *Nature* 290, 19–152
10 Marescaux, C. *et al.* (1992) $GABA_B$ receptor antagonists: potential new anti-absence drugs. *J. Neur. Transm.* 35 S179–S187
11 Bormann, J. (2000) The 'ABC' of GABA receptors. *Trends Pharmacol. Sci.* 21, 16–19
12 Gottesmann, C. (2002) GABA mechanisms and sleep. *Neuroscience* 111, 231–239
13 Gottesmann, C. *et al.* (1998) The intermediate stage of sleep and paradoxical sleep in the rat: influence of three generations of hypnotics. *Eur. J. Neurosci.* 10, 109–414
14 Gauthier, P. *et al.* (1997) Influence of a $GABA_B$ receptor antagonist on sleep-waking cycle in the rat. *Brain Res.* 773, 8–14
15 Arnaud, C. *et al.* (2001) Study of a $GABA_C$ receptor antagonist on sleep-waking behavior in rats. *Psychopharmacology (Berl)* 154, 415–419
16 Sakai, K. and Cochet, S. (2003) A neural mechanism of sleep and wakefulness. *Sleep Biol. Rhyt.* 1, 29–42
17 McGinty, D.J. *et al.* (1974) Neuronal unit activity and the control of sleep states. In: *Advances in Sleep Research* Vol. 1 (Weitzmann, E., Ed.) pp. 173–216, Spectrum
18 Hobson, J.A. *et al.* (1975) Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. *Science* 189, 55–58

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HPLC–NMR–MS: past, present and future

The development of technology to enable a practical and useful direct coupling of HPLC with NMR spectroscopy to get real-time detection and identification of the eluting compounds is an interesting story [1]. It is a case of waiting for technology to catch up with demand. Early studies were conducted in the late 1970s and early 1980s; these included the concept of placing the HPLC column inside the NMR superconducting magnet [2–4]. However, these failed, largely as a result of technical limitations. It was the advent of higher-field NMR magnets and digital electronic technology that improved NMR sensitivity and, coupled with improvements in methods for suppressing the large NMR peaks from solvents, the methodology became viable and commercial instruments appeared on the market.

The desire for real-time analysis led to initial studies of HPLC–NMR using the continuous flow approach. This led to poor detection limits and it soon became clear that stopped-flow approaches, where the entire armoury of NMR tools for molecular identification could be used, was the way forward. Later, in 1995, the first attempt to combine HPLC with both NMR and MS was published [5]. Then, the debate ensued as to whether the NMR and MS should be in series or in parallel; the parallel arrangement soon became the norm because it enabled either the NMR or the MS to be used as the intelligent detector in finding eluting peaks of interest. For example, if the eluting compound contains fluorine, then ^{19}F NMR spectroscopy would be a highly selective approach for detecting fluorinated molecules with no background. By contrast, if the molecule of interest contains a well-defined molecular fragment, for example, a sulfate conjugate of a drug (a typical drug metabolite), then searching for a mass loss of 80 amu is, again, a selective and sensitive MS approach. Thus, more recently, the stopped-flow method with NMR and MS detection in parallel has become standard.

More recently, the idea of trapping the eluting peaks into capillary loops for later off-line analysis has gained much support and is increasing in usage; commercial instruments for performing this process are now available. This process can select up to 36 peaks from a running chromatogram and later conduct all necessary NMR and MS analyses under computer automation. This is of great benefit to industrial laboratories, such as those performing drug metabolite identification in the pharmaceutical industry.

Several further developments should not be forgotten. For example, it is possible to hyphenate even more techniques into a total combined analysis system (hypernation) [6]. One way in which this has good practical use is to combine more than one type of MS into the analytical system. Methods that are beginning to be used include inductively-coupled plasma (ICP) MS, which causes all molecules to be reduced to their atomic content, enabling the assay of a wide variety of heavy atoms. For a drug molecule that contains chlorine and is extensively metabolised in the body, a single HPLC–ICPMS run will detect all of the chlorinated molecules in a sample. Such a 'chloratogram' can be a useful tool for identifying both the number and quantity of such metabolites. If the HPLC analysis is then combined with a high resolution MS system, such as a time-of-flight or a Fourier transform MS, then masses can be determined highly accurately, leading directly to the elemental formula [7].

Further developments are occurring in the NMR component of HPLC–NMR–MS, including the development of NMR detectors that are cooled to cryogenic temperature. This causes a thermal noise reduction of ~500% and, hence, results in substantially improved signal:noise ratios, with a consequent impact on detection limits. Combining such developments with on-going attempts at miniaturization [8] will see the old adage of NMR being an insensitive technique stamped out forever.

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