213P CARDIOVASCULAR REGULATION: IMPORTANCE OF THE LOCUS COERULEUS

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Supramedullary and medullary structures are involved in blood pressure regulation. In these brain areas, several transmitters released from their neurons may either increase or decrease blood pressure thus contributing to blood pressure homeostasis (Philippu, 1988; Singewald & Philippu, 1996). The interest of this study is focused on the role of the catecholaminergic and serotonergic innervation of the locus coeruleus (LC) in cardiovascular control.

The LC was superfused through a push-pull cannula (Philippu, 1985) with artificial cerebrospinal fluid and the neurotransmitters noradrenaline (NA), dopamine (DA), serotonin (5-HT), as well as 5-hydroxyindoleacetic acid (5-HIAA) were determined in the superfusate.

Previous studies have shown that, in the cat, baroreceptor activation by phenylephrine decreases the release of NA in the LC. Vagotomy combined with transection of the aortic depressor nerves abolishes the effects of phenylephrine on NA release. Since, on the other hand, the selective stimulation of baroreceptors in the carotid sinus by an inflatable catheter does not influence the release of NA in the LC, noradrenergic neurons within the LC seem to respond predominantly to impulses originating from baroreceptors of the aortic arch and the cardiopulmonary system (Schneider *et al.*, 1995).

The involvement of the LC in the chemoreceptor reflex was investigated in cats and Sprague-Dawley rats. In the cat, chemoreceptor stimulation by intracarotid infusion of CO2-saturated $NaHCO_3$ (CO_2-$NaHCO_3$) increases blood pressure (18±1 mm Hg, $P<0.05$, n=8) and enhances the release of NA in the LC (145±5%, $P<0.05$). Carotid body denervation abolishes the CO_2-$NaHCO_3$-induced release of NA. Since superfusion of the LC with CO_2-$NaHCO_3$ does not influence either blood pressure or the release rate of NA, the findings show that impulses originating from chemoreceptors of the carotid body stimulate catecholaminergic neurons the LC. In Sprague-Dawley rats, chemoreceptor stimulation by intravenous administration of KCN (30 µg/min) also enhances the release of 5-HT in the LC (158±6%, n=10, $P<0.05$). As in the cat, sinoaortic denervation of the rat abolishes the KCN-induced release of serotonin, thus suggesting that the activity of serotonergic neurons within the LC is triggered by peripheral chemoreceptor activation.

In 10- to 12-week-old normotensive WKY rats (n=11) the basal release rates of 5-HT and 5-HIAA in the LC are 1.3±0.2 and 83.7±15.2 fmol/min respectively and the arterial blood pressure 103±1 mm Hg. In spontaneously hypertensive rats (SHR; blood pressure 140±1 mm Hg, n=10) the release rate of 5-HT is greatly enhanced (2.9±0.4 fmol/min, $p<0.01$). The extracellular concentration of 5-HIAA does not differ from that in WKY rats. Experimentally-induced increases in blood pressure enhance the release of 5-HT in the LC while falls in blood pressure exert the opposite effect, suggesting that serotonin released in the LC possesses a counteracting, hypotensive function. Hence it seems that the enhanced release of 5-HT in the LC of genetically hypertensive rats reflects a mechanism counteracting the disturbed blood pressure homeostasis. On the other hand, the release rates of NA and DA are similar in WKY rats and SHR. In contrast to 5-HT, increases in blood pressure decrease, while decreases in blood pressure enhance the release rates of NA and DA in the LC of WKY rats and SHR.

Taken together, the findings show that, in the LC, noradrenergic and serotonergic neurons are involved in the baroreceptor chemoreceptor reflexes and the maintenance of cardiovascular homeostasis.

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214P REGULATORY FUNCTIONS OF AMINES IN THE CNS: MOOD AND DEPRESSION

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The role that brain 5-HT plays in the regulation of mood is far from clear. However, it is now becoming obvious that brain 5-HT plays an important role in both the drug therapy of mood disorder, and also the cause of mood disorder in vulnerable individuals. This talk will focus on some of the recent advances in 5-HT pharmacology, and illustrate how these advances are being applied to further our understanding of mood disorder and improve its treatment.

All current antidepressant medications take several weeks of administration to reach their full therapeutic effect. It is commonly believed that this delay in onset of therapeutic effect is explained by neuroadaptive changes in brain function that are linked to alterations in gene expression and triggered by some of the acute pharmacological effects of the treatments. Several lines of evidence suggest that an increase in 5-HT function is the trigger for many of these neuroadaptive changes.

Firstly, in microdialysis experiments it is clear that many (but not all) types of antidepressant drug increase extracellular levels of 5-HT. A combination of animal and human data suggest that this effect often occurs in response to acute antidepressant drug treatment but becomes greater during the course of administration. This delayed rise in extracellular 5-HT may well be due to a gradual desensitisation of 5-HT_{1A}, and possibly 5-HT_{1B}, autoreceptors.

Secondly, many types of antidepressant drugs which extracellular 5-HT, including those that are selective inhibitors of 5-HT uptake (SSRIs), commonly evoke changes in expression of specific genes. These changes often come about following repeated but not acute antidepressant drug administration, and include genes for certain transmitter receptors, neurotrophic factors and neuropeptides. Moreover, these effects are region specific and some are localised to mesocorticolimbic areas that recent neuroimaging studies suggest are abnormal in major depression.

Thirdly, emerging but not yet complete evidence from basic studies using 5-HT receptor selective ligands suggests that activation of 5-HT_2 receptors and possibly other 5-HT receptor subtypes ($5\text{-HT}_{4,6,7}$) leads to some of the same changes in gene expression induced by the antidepressant drug treatments.

Whilst the clinical relevance of many of the neuroadaptive changes induced by antidepressant treatments is not certain, therapeutic advances are being made by strategies aimed to accelerate the effect of antidepressants on 5-HT function. Specifically, basic studies show that the effect of an SSRI on extracellular 5-HT can be markedly enhanced by antagonism of 5-HT_{1A} autoreceptors, and even more so by combined 5-HT_{1A}/5-HT_{1B} blockade. New findings using immediate early genes as markers of increased neural activity establish that these effects lead to increased 5-HT function at the postsynaptic level. There are now several double blind trials reporting that SSRI augmentation with the 5-HT_{1A}/β-blocker pindolol, speeds up the onset of antidepressant action. However, negative findings in some clinical studies, and new evidence that pindolol has partial 5HT_{1A} agonist properties, suggest that the treatment strategy is not yet optimised. Ongoing studies in both animals and humans are exploring optimal ways of SSRI augmentation based on 5-HT autoreceptor blockade and other strategies.

Overall, it is clear that the neuroadaptive changes in brain function induced by antidepressant treatment are complex and that this reflects in part the diversity of 5-HT pathways and 5-HT receptor subtypes. Combined preclinical and clinical studies are leading to a more complete knowledge of these neuroadaptive changes, and this will promote the rational development of quicker acting and more effective antidepressant treatments.

215P SEROTONERGIC MECHANISMS AND FEEDING BEHAVIOUR

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Serotonin (5-HT) has been implicated in the control of feeding and body weight gain. Drugs increasing extracellular 5-HT by releasing or inhibiting uptake of 5-HT have been shown to reduce food intake. Drugs inhibiting 5-HT activity, e.g. by inhibition of firing rate of 5-HT neurones, have been demonstrated to affect also food intake. The hypothalamic nuclei play a crucial role in the control of ingestion by 5-HT mechanisms. Food intake or deprivation decreases or increases, respectively, 5-HT levels in this area.

A feedback loop relating hypothalamic 5-HT and food intake has been first proposed by Leibowitz *et al.* and later confirmed in *in vivo* studies by Schwartz and Hoebel. This hypothesis implicates that reduced 5-HT function after lesion of raphe nuclei may provoke hyperphagia and increase body weight. In our studies the median raphe nucleus and/or the dorsal raphe nucleus were lesioned with 5,7-DHT and food intake in food-deprived rats as well as in freely fed rats at the beginning of the dark phase was determined 1, 3, and 4 weeks after lesion. Lesioned rats did not show any change in food intake within an observation period of 24 h or in their weight gains compared to sham lesioned rats.

In contrast to our expectations based on markedly decreased 5-HT content in relevant brain structures, including the hypothalamus, the results did not reveal any influence of a long lasting decreased 5-HT activity in the CNS. Additionally, pharmacological effects of the 5-HT_{1A} agonist 8-OH-DPAT and the 5-HT releasing drug fenfluramine did not differ between median raphe lesioned andsham lesioned rats. Further, rats pretreated with the 5-HT depletor PCPA showed only a significant decrease in food intake up to two days after last administration, whereas this effect diminished in the following days. Likewise, in transgenic rats with downregulation of tryptophan hydroxylase activity by expression of antisense against RNA of this enzyme, a transient decrease in feeding and weight gain as well as a stronger inhibition of food intake after fenfluramine were observed in the male heterozygotes.

Long lasting changes in 5-HT activity appeared insufficient to elicit long lasting effects on feeding and weight gain. Deficiencies in 5-HT function seem to be compensated. To investigate further the complex relationship between 5-HT and ingestion, microdialysis studies in freely moving rats were performed. 8-OH-DPAT is known to reduce central 5-HT release, including the lateral hypothalamus, and to induce food intake in freely-fed rats. In contrast, in hungry rats 8-OH-DPAT suppressed food intake. A hypothalamic 5-HT decrease could be confirmed in freely feeding rats, whereas the same dose of 8-OH-DPAT had no effect on 5-HT release in food deprived rats. Several experiments indicate that the state of satiety is reflected by changes in 5-HT turnover, with elevated tryptophan in the hypothalamus of food deprived rats. Therefore, it is suggested that an acute decrease in 5-HT as induced by 8-OH-DPAT can be overcome by another state dependent and dominant process. This is supported by the failure of 8-OH-DPAT to increase food intake at the beginning of the dark phase when circadian 5-HT activity is high.

The results of our studies support the suggestion that at least some of the long time changes in 5-HT activity can be functionally compensated, whereas short time changes may result in more striking effects on feeding and both short and long term 5-HT changes may interfere.

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216P BRAIN AMINES AND AVERSION

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Aversive behaviours involve both unconditioned and conditioned responses: a good example is the distinction between a panic attack and panic disorder. The former is an unconditioned response involving activation of the brain aversive system, in particular the periaqueductal grey (PAG) but also the amygdala. Panic disorder, however, involves conditioning associated with concern as to when the next attack will occur and thus implicates brain regions important in anticipatory behaviour such as the amygdala and the hippocampus. The use of appropriate animal models will help identify the role of specific amines and their receptors in unconditioned and conditioned aversive behaviours.

Rats emit ultrasound at different frequencies depending on the nature of the stimuli. Exposure to predators results in calls within the 18-27KHz range; these elicit defensive and escape behaviour. We have shown that exposure of naive Lister hooded rats to 20KHz ultrasound produces a marked initial escape response followed by freezing, but with Wistar rats only a freezing response occurs. The behavioural response is associated with increased c-fos immunoreactivity in the dorsal PAG in the Listers but in the ventral PAG in the Wistars. This result suggests a strain difference not only in the behavioural response but also in the neural pathways involved. This view is further substantiated by studies showing that the escape response (dorsal PAG) in the Listers is augmented by 5,7dihydroxytryptamine (5,7-DHT) lesions of the serotonergic neurones in the dorsal raphe while the same lesion had no effect on the freezing response (ventral PAG) in the Wistars.

Furthermore, injection of the 5-HT_{1A} agonist 8-OHDPAT into the dorsal PAG inhibited the ultrasound induced response in the Listers. In contrast, 6-hydroxydopamine (6-OHDA) lesions of the noradrenergic locus coeruleus had no effect on the escape response but decreased the duration of the freezing behaviour.

Conditioned aversive behaviour is observed using a conditioned emotional response (CER) paradigm when previous footshock produces freezing behaviour on re-exposure of the rat to the contextual cue; an effect reduced by noradrenergic lesions. Microdialysis studies have shown exposure to the contextual cue to increase dopamine but not serotonin release in the nucleus accumbens, while serotonin release is increased in the ventral hippocampus. Activation of 5-HT_{2C} receptors in the amygdala causes pro-aversive responses. Rats reared in isolation show an enhanced behavioural response to the contextual cue, potentiation of aversion induced increase in dopamine release in the accumbens but inhibition of the increase in hippocampal serotonin release.

The results suggest different functional roles for different amines in different brain regions in aversive behaviours. Noradrenaline is involved in attention and aversive learning rather than being a principle activating switch. Serotonin in the dorsal PAG, probably via 5-HT_{1A} receptors, restrains acute fear responses while, in the hippocampus, increased serotonin release is concerned with coping with aversive situations. In contrast, activation of 5-HT_{2C} receptors in the amygdala increases aversion. Clinical anxiety may occur when the relative importance of specific components of these complex neuronal interactions become distorted, normal balance is disrupted and SSRIs may act to restore the balance.

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Drug dependence is an extremely complex phenomenon. Although a number of relevant phenomena have been successfully studied, many questions remain to be solved. Various phenomena seem to interact and to contribute to drug dependence.

There is evidence that enhancement in dopaminergic neurotransmission, in particular in the mesolimbic dopaminergic pathways, seems to be related to addictive properties of psychomotor stimulant drugs, opioids, nicotine and perhaps ethanol, whereas addictive properties of sedative-hypnotics (such as benzodiazepines) are probably not directly related to dopaminergic activation.

Increases in dopaminergic neurotransmission seem at first glance to be the cause of rewarding properties of drugs or other manipulations leading to a feeling of "high". However, this assumption is probably too simplistic. There is more evidence that dopaminergic activation in the nucleus accumbens is related to anticipatory responses to reinforcing stimuli. Dopamine might modulate signals of reward and enhance approach behaviour to conditioned stimuli related to reinforcement.

Withdrawal from psychomotor stimulants or opioids leads to decreases in dopaminergic neurotransmission, which might be related to dysphoria and by this again lead to drug-taking. Withdrawal symptoms, as signs of physical dependence, are relatively short-lasting, however, whereas the vulnerability in addicted patients is very long-lasting. Accordingly, withdrawal does not seem to be the most relevant factor in addiction, although it might in many cases exacerbate the problem.

Sensitisation after repeated treatment was found in psychostimulant drugs (cocaine, amphetamine) and in opioids. This phenomenon in particular reflects enhanced signs of dopaminergic neurotransmission and is very long-lasting even after withdrawal of the drug. There is some evidence that the dopaminergic neurotransmission is further enhanced when animals are again treated with a test dose of the drug (e.g. cocaine) after repeated treatment, when compared with the effects of the first dose.

However, not all results seem to agree with this assumption and the mechanisms might be drug- and schedule-dependent. In our own studies it was found that behavioural sensitisation to cocaine was, under the protocol used, not related to any visible alterations in nigrostriatal or mesolimbic dopamine release, alterations in mRNA of tyrosine hydroxylase in midbrain or alterations in striatofugal pathways (mRNA for preprodynorphin labelling the "direct pathway" and for preproenkephalin labelling the "indirect pathway"). Under different conditions, dopaminergic mechanisms might be more relevant in sensitisation phenomena. Conditioning processes clearly can contribute to sensitisation, but even in the apparent absence of conditioning phenomena, sensitisation can be observed.

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218P BEHAVIOURAL AND NEUROCHEMICAL EVIDENCE FOR A ROLE OF CORTICAL ACETYLCHOLINE IN VISUAL ATTENTION

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Recent evidence suggests that the cholinergic projections that arise from the basal forebrain to provide diffuse projections throughout the neocortex play a prevalent role in attentional processing.

Infusions of the cholinergically selective immunotoxin 192 IgG-saporin (SAP) into the area of the nucleus basalis magnocellularis/ substania innominata (nbm/SI) of rats was found to produce profound loss of cortical cholinergic innervation as indicated by a striking loss of AChE positive fiber staining. Animals with these severe lesions were significantly impaired at baseline conditions in a successive discrimination, sustained attentional task. These behavioral impairments were persistent over an extended period of retraining and were unchanged after the administration of drugs aimed at ameliorating these deficits including physostigmine and FG 7142.

More recently the effects of similar cholinergic lesions were tested in a 5-choice serial reaction time task (CSRTT). Lesioned animals tested in this task showed significant but small impairments under baseline conditions than formerly shown. Additional testing was performed under conditions of increased attentional demands by changing in event rate, event asynchrony and stimulus duration. Lesioned animals were found to be severely impaired under conditions of an increased event rate (See Figure 1).

Following probe implantation into the frontal cortex, *in vivo* microdialysis was conducted while animals performed in the S CSRTT under challenge conditions to determine the changes in cortical cholinergic efflux following these increases in the attentional demands. Histological verification of the extent of the lesions was obtained by staining tissue for choline acetyltransferase (ChAT), and acetylcholinesterase (AchE). These assessments revealed a marked loss of the magnocellular neurons of the basal forebrain with corresponding loss of cholinergic innervation throughout the cortex.

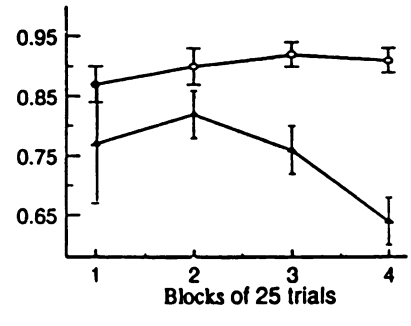

Figure 1. Sham-lesioned (open circles) animals and lesioned animals (closed triangles) tested with an increased event rate. Performance is shown over the course of 100 trials divided into blocks of 25 triaS/each (abscissa) allowing a determination of changes in percent correct (ordinate) with increased time on task.